Chapter 1

1. GENERAL INTRODUCTION

1.1 Australian Transportation Practices and associated challenges

The ability of the Australian beef industry to demonstrate the welfare status of cattle during land transport is difficult as there is little scientific evidence of the well-being of cattle transported under Australian conditions and practices. There are some distinct differences in Australian environmental conditions and transport practices compared to other countries. In particular Australian livestock can be transported long distances under hot and dusty conditions, particularly for cattle being moved throughout northern Australia. Previous Australian livestock transport research conducted over 20 years (Wythes et al., 1981, 1984, 1985) ago focussed primarily on the impact of transport on meat quality. However, whilst important, the results do not provide either a complete or accurate indication of animal welfare during long distance transport.

Transport practices in Australia are underpinned by a Model Code of Practice for the Welfare of Animals (SCARM, 2002). However, the current sheep and cattle codes represent minimum standards and are not prescriptive. In Australia, the codes of practice for the land transport of sheep and cattle (SCARM, 2002) stipulates that mature livestock should not be transported by road for more than 36 hours without a break of at least 12 hours off the vehicle during which time feed and water are to be provided. It is also documented in the code of practice that this maximum duration can be extended to 48 hours if the stock are not exhibiting signs of fatigue or distress and poor weather conditions are not predicted and the journey can be completed within 48 hours. If stock are to be

transported for durations of up to 48 hours, they must be rested with access to feed and water for a minimum period of 24 hours subsequently.

1.2 Livestock Transport in the European Union

In recent years in some European countries such as Great Britain, farmers and consumers have been questioning farm animal welfare during transport (Le Neindre et al., 2001). In a communication from the Commission to the Council and European Parliament on the Protection of Animals during Transport (2002) it is reported that road transport was the most significant mode of movement for live animals in the European Union (EU). The EU has paid specific attention to journeys more of than 8 hours. Le Neindre et al. (2001) lists the main concerns of the regulations as follows:

1. Density of the animals, for each type of animal, space requirements are given.
2. Design of the facilities (loading and unloading) and of the truck in particular, the animals should be able to drink either during the whole journey (pigs) or during stops (cattle, sheep and goats). The latter group should also be allowed to eat during stops; the floor should be bedded; the temperature should range between 5 and 30°C, naturally ventilated $\pm$ 5°C inside the vehicles, depending on the outside temperature.
3. Duration of the journey, maximum length of journeys without unloading are given (e.g. 19 h for unweaned animals, 24 h for pigs, 29 h for cattle), adult cattle and sheep should have a stop of 1 h after a 14 h and then the journey can resume for another 14 h. After the last period, animals should be unloaded for at least 24 h in

specific resting areas before further transport can be undertaken. During this period, the animals should be fed and watered. (Table 1.2.1)

4. All companies should have a license that can be withdrawn if they do not comply with the requirements.

Revisions to maximum travelling times and animal stocking densities on vehicles were proposed in November 2004. However, the EU countries did not reach an agreement on these proposed revisions (European Commission, 2005). There were some concerns to reduce the journey time for sheep and cattle from 14 hours down to 9 hours and the European Commission has agreed that these two issues will be the subject of a further separate proposal to be presented no later than 2007.

Table 1.2.1 Maximum Duration of transport of farm animals in trucks (Directive 95/29/CE)

Species	Transport (first part), h	Resting on board, h	Transport (second part), h	Resting out of the truck, h
Calves, lambs, piglets	9	1 (water and feed)	9	24
Pigs	24 (water)			24
Cattle and Sheep	14	1 (water and feed)	14	24

(Source: Le Neindre et al., 2001)

In November 2004, updated regulations regarding animal transport in the EU (European Commission, 2005) were introduced with the aim of improving accountability by identifying "who is responsible for what" during livestock transport.

The introduction of the new rules (European Commission, 2005) aims at dealing with situations before and after transport. The enforcement of animal transport rules across Europe will ultimately require monitoring tools such as checks via satellite navigation systems from 2007. There are likely to be stricter rules on journey durations of more than 8 hours which could involve substantial upgrading of vehicle standards and equipment. It is speculated that the regulation will probably introduce a ban on transporting very young animals (ie calves < 10 days of age, pigs < 3 weeks and lambs < 1 week), except if the journey is less than 100 kilometres. The transport of calves < 14 days of age on journeys exceeding 8 hours will probably not be permitted.

Pregnant female animals will not be considered fit for transport if they have reached the last 10% of gestation and for a period of one week after giving birth (Commission of the European Communities, 2002).

1.3 Livestock Transport in North America

The majority of beef cattle raised in the United States of America (US) will undergo transportation at least once in their life time (Swanson and Morrow-Tesch, 2001) and the livestock industry's current infrastructure deems transportation a necessary component of most operations (Speer et al., 2001).

In the US, there is a tendency towards centralization of modern slaughter facilities, with corresponding increases in transportation times (Speer et al., 2001) and although transport distances between feedlots and slaughter plants are relatively short, weaned calves and

yearlings often have to travel 1000-3000 km to feedlots (Tarrant and Grandin, 2000). In the US there are no rest-stop period requirements. However, in Canada, a rest stop is required after 48h of travel (Tarrant and Grandin, 2000). It is argued that, in practice, unless resting facilities are adequate and the animals are unloaded carefully, rest stops may be counter productive and only serve to prolong the overall journey time and increase stress (Tarrant and Grandin, 2000).

In the US there are no federal laws in place documenting codes of practice for transportation of livestock although there are a number of handbooks that have been published outlining best practice for preparing animals for transport, the design of transport vehicles and their preparation to carry animals (i.e. bedding and ventilation), loading, unloading and stocking densities. One of the most critical considerations with regard to stocking density appears to be weather conditions, particularly during cold weather, where increased stock density is more desirable (Tarrant and Grandin, 2000).

The Livestock Conservation Institute (now the National Institute for Animal Agriculture) (Speer et al., 2001) produced the *Livestock Trucking Guide*. Grandin (1992) lists the essential elements of livestock transport in the US in the Livestock Trucking Guide as:

1. Appropriate vehicle selection contributes to maintaining contentment of stock whilst on the road.
2. Proper vehicle preparation helps to prevent injury and disease transmission during transport.

3. During hot weather, livestock should be hauled at night or early morning.
4. Discourage mixing of unfamiliar animals to minimize the effects of behavioural stress and injuries that may result from fighting.
5. Movement of wet animals during cold weather should be avoided to prevent deaths due to wind chill.
6. Livestock should be unloaded and rested if the trip will last more than 48 hours.
7. Careful, quiet handling that keeps animals calm will reduce stress.
8. Proper load density given various weather environments should be used.
9. Non slip truck flooring and an air ride suspension will help reduce stress.
10. Load cattle quietly and move them at a walk or trot. Eliminate electric prods and loud noises.
11. Smooth starts and stops will help prevent bruises, stress and weight losses.
12. Responsible care during transit helps to maintain health of stock.
13. Proper unloading practices help to prevent injury and further stressing of already tired animals.
14. Good post transit care helps assure successful highway movement by preventing injury and helping stock to recover from the rigidity of transport

1.4 Conclusion

Although livestock in Australia appear to be well adapted to our environmental conditions, the differences between our practices and those of other countries where livestock can be transported over significant distances, such as the US and the EU, suggest that more detailed investigations are required. If Australian practices and regulations are going to be

different from other countries then it is necessary to gather objective evidence of the animal welfare outcomes during transport of livestock in Australia. The aim of this research is to address this issue where the impact of two transport practices is investigated, specifically, the effects of different loading practices and transport duration on the physiological response in cattle.

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Chapter 2

2. LITERATURE REVIEW

2.1 Introduction

The Australian livestock industry relies heavily on the movement of livestock by road transport. This is a necessary and common practice for livestock operations. The magnitude of livestock transportation has never been quantified, however there are approximately 23 million cattle in Australia, most of which travel at least once on a truck in their lifetime, depending upon the production system and market destination. This equates to at least 20 million cattle being transported across Australia annually, with potential numbers of 40 million, if the movement of store and feedlot cattle are taken into account where two to three journeys are common. With numbers of this order, road transport of livestock has both significant animal welfare and economic implications. The impact that transport has on animal welfare is not well understood particularly under Australian transport conditions. It is therefore paramount that the welfare outcomes of Australian livestock transport practices are quantified.

During transport, animals undergo major changes in their physical and social environments which may elicit stress that may exceed that associated with normal management practices (Fraser, 1979). Unfortunately, there is very little objective information in Australia on how animals respond to the various stressors that apply during road transportation (Eldridge et al., 1988).

The main concerns related to livestock transport can be consolidated into four key points (Hartung et al., 2003). Transport can cause severe stress in animals resulting in poor welfare and possible mortality. Stressful transport may have a negative effect on carcase and meat quality. There is a risk of the spread of infectious disease among animals during transport and at their final destination, and transport may have a negative impact on the environment by emissions from the stock and the vehicle engines. A combination of these concerns necessitates an understanding of how ruminants respond to the stressors that apply during transport. Moreover, it raises the question as to how we can best measure and understand the behavioural and physiological changes that occur during transport in order to determine if animal welfare is being compromised. It is important to note that if animal welfare is shown to be compromised during specific transport practices, it is imperative to make improvements that minimise the biological cost to the animal, thus achieving better welfare outcomes.

Long distance livestock transport is rapidly gaining prominence on the international animal welfare agenda. The World Society for the Protection of Animals (WSPA) who are about to promote an international campaign, spending at least 3 million dollars targeting long distance transport over the next twelve to eighteen months (H.Wirth 2005 pers comm., 24 May). Australian practices will undoubtedly be scrutinised given the vast distances, durations and numbers of animals that are transported within and across the continent annually.

There is a scarcity of scientific literature examining Australian road transport practices and what impact they have on animal welfare. However, there is an extensive amount of literature investigating the effects of transport on cattle (see review by Knowles, 1999), though, the majority of research has been conducted within the European Union and to a lesser extent in the United States. Whilst it is a significant body of work, it is apparent that this research does not necessarily address specific issues relevant to Australian transport conditions and practices such as duration.

2.2 General physiological response in ruminants

In general terms, stress can be defined as a physiological disturbance and/or a biological response and occurs when animals are exposed to noxious stimuli or threatening challenges (Gregory, 2004). Significant stress can overtax control systems, reduce fitness and ultimately, compromise the well-being of the animals if adaptation does not occur (Broom, 2003[a]). When stress is severe, homeostatic processes are put under abnormal pressure, as the animal attempts to maintain homeostasis through physiological or behavioural adjustments (Gregory, 2004; Knowles and Warriss, 2000). The more an animal has to expend, in its efforts to adapt with a situational or environmental challenge, the higher the psychological and biological cost. Consequently this will lead to a compromise of its welfare (Knowles and Warriss, 2000).

According to Moberg (2000), the physiological response can be divided into three general stages:

1. The recognition of a stressor

2. Activation of the biological defence mechanisms

3. The consequence of the physiological response

A physiological response **begins** with the central nervous system (CNS) perceiving a potential threat to homeostasis (Moberg, 2000). If the threat is significant, a biological response is initiated that consists of a combination of four basic defence responses which are shown in figure 2.2 (Moberg, 2000).

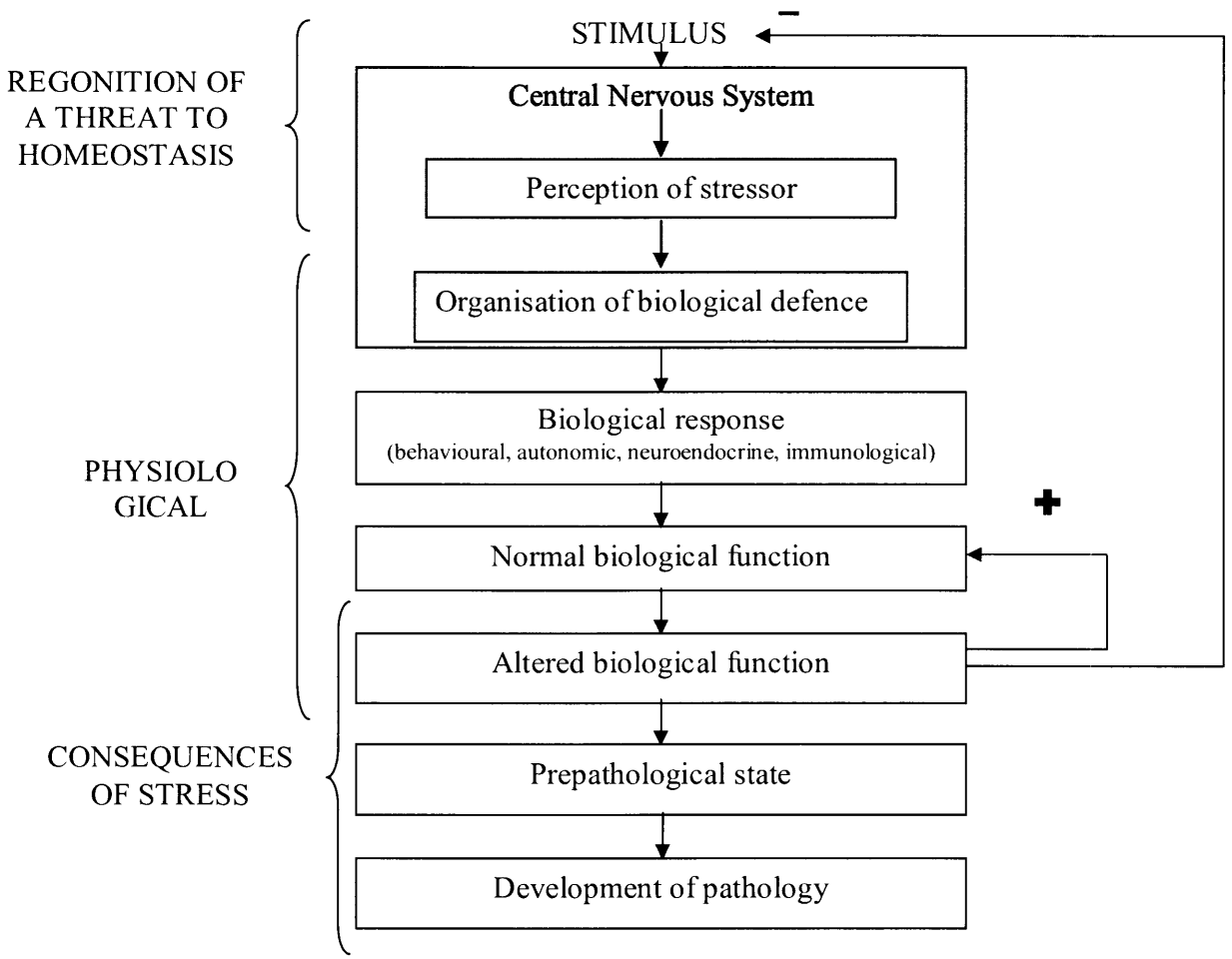


Figure 2.2 A model of biological response of animals to stress (Moberg, 2000)

The basic biological responses include autonomic, neurological, immunological and/or behavioural changes (Moberg, 2000; Siegel and Gross, 2000).

The first line of defence that generally occurs when an animal attempts to adjust to a challenge is to alter its behaviour (Moberg, 2000). An animal may achieve this by implementing a behavioural adjustment in response to a stressor and it may simply include the animal removing itself from a threat, e.g. seeking shade on a hot day (Moberg, 2000). During transport when animals may be confined, the behavioural actions that an animal wishes to make are significantly limited. How this impacts upon the severity of the animal's overall physiological response is not well understood (Ladewig, 2000).

The second mechanism activated in response to stress is the autonomic nervous system (ANS) also referred to as the "fight or flight" response (Tortora and Grabowski, 1993; Moberg, 2000; Fraser and Weary, 2004; Webster, 2005). Changes in the ANS have consequential effects on a number of other biological systems namely the cardiovascular and gastrointestinal systems, the exocrine glands and the adrenal medulla which secrets adrenaline and noradrenaline (Moberg, 2000). When the "fight or flight" response is activated, sequences of nerve cell firing occur and adrenaline and noradrenaline are released into the bloodstream (Tortora and Grabowski, 1993). When these hormones are released it results in tachycardia , increased blood pressure, opening of the airways in the lungs, slower digestion, redirection of blood flow to major muscle groups, and changes in various other autonomic nervous functions. These changes are designed to provide the animal with a burst of energy and strength (Tortora and Grabowski, 1993). Because the

autonomic responses affect very specific biological systems and the effects are of relatively short duration, this can generally be considered as more of an acute physiological response.

The third biological response is that of the neuroendocrine system and it involves the activation of the hypothalamic-pituitary-adrenal (**HPA**) axis (Fraser and Weary, 2004), which can have a broad, long-lasting effect on several biological systems (Moberg, 2000). For example, behaviour and key biological systems such as the immune, reproduction and metabolic systems are regulated by stress-mediated pituitary hormones (Moberg, 2000).

In research aimed at determining the physiological responses in livestock, researchers have tended to concentrate on the HPA axis (Lay et al., 1996). Activation of the HPA axis initiates the release of corticotrophin releasing hormone (CRH) from the hypothalamus, which stimulates release of adrenocorticotropic hormone (ACTH) from the anterior pituitary into circulation, which in turn, stimulates secretion of glucocorticoid hormones (such as cortisol) from the adrenal cortex (Fraser and Weary, 2004).

The immune system is modulated by activation of the HPA axis (Moberg, 2000). One of the main roles of the HPA axis is in combating disease (Gregory, 2004). Cytokines that are produced in response to disease vectors stimulate ACTH secretion from the pituitary, partly through activating CRH release, resulting in release of glucocorticoid hormones. Glucocorticoids also reduce inflammation, preventing the spread of disease and promote supply of substrates required for repair and recovery (Gregory, 2004). The duration of stress can impair the capacity of the immune system to respond to glucocorticoid hormones

that are normally responsible for terminating an inflammatory response following infection and/or injury (Gregory, 2004). Therefore, measurements of the change in immune function are one of the more useful indicators for evaluating the biological cost to the animal in response to a stress challenge. These immunological measures are discussed in more detail in section 2.4.1.

These changes in biological responses can be used to quantify the impact of transport stress (Tarrant, 1990). Measurement of the magnitude of behavioural and/or physiological adjustments in response to stressful challenges has provided a useful basis to underpinning the assessment of animal welfare (Knowles and Warriss, 2000).

2.3 Stressors that apply during livestock transport of ruminants

Many factors are involved in the complex issue termed "transport stress" (Swanson and Morrow-Tesch, 2001). The most aversive factors associated with livestock transport are the processes of loading and unloading, handling, unskilled and inappropriate driving, poor road conditions, fluctuations in environmental conditions, insufficient ventilation (impacted by dust and exhaust fumes), high stocking densities, mixing of unfamiliar animals, deprivation of food and water, vehicle vibration and motion and journey duration (Eicher, 2001; Hartung et al., 2003; Kent and Ewbank, 1983; Knowles, 1999). However, the responses to such stressors are also influenced by the complex interaction between genetics and previous experience (Grandin, 1997; Broom, 2003[a]), and therefore are sometimes difficult to predict.

Different combinations of stress produce mixed physiological and psychological responses (Swanson and Morrow-Tesch, 2001). The responses in some instances will be relatively short-lived (acute) whereas others will be more moderate or chronic in nature and may take longer to develop during a transport event. More importantly, the intensity combined with the duration of the stressor(s) will ultimately determine the biological cost to the animal. For example, the process of mulesing sheep is of short duration but the intensity of this process is relatively severe, particularly from a pain threshold perspective. Whilst acute stress is short lived, if the duration and intensity is large, this is a cause for concern from an animal well-being viewpoint.

Many stimuli that are considered to be stressful activate the HPA axis (Jacobson and Cook, 1998; Lay et al., 1996; Swanson and Morrow-Tesch, 2001), and during long-term transportation, animals can be exposed to repeated stressful events during a journey such as having to compete for space with other animals in close confinement. In situations of continuous exposure to such stressors during transport, activation of different hormonal pathways may cause biochemical changes in target tissues (Odore et al., 2004).

The pituitary adrenal axis is also activated during a physiological response, with an associated increase in the circulating levels of cortisol, glucose and free fatty acids (Knowles, 1999; Swanson and Morrow-Tesch, 2001). Measures of cortisol, changes in heart rate, blood composition (electrolytes, hormones, metabolites and enzymes) and liveweight are some of the physiological indicators used to assess the response of livestock to transport (Tarrant, 1990).

Figure 2.3 illustrates the different stressors that can apply during a long transport duration event of 48 hours. Animals that are subjected to journeys of long duration are at a greater risk of fatigue, dehydration and tissue catabolism which may ultimately increase the biological cost to the animal during transport, up to 48 hours.

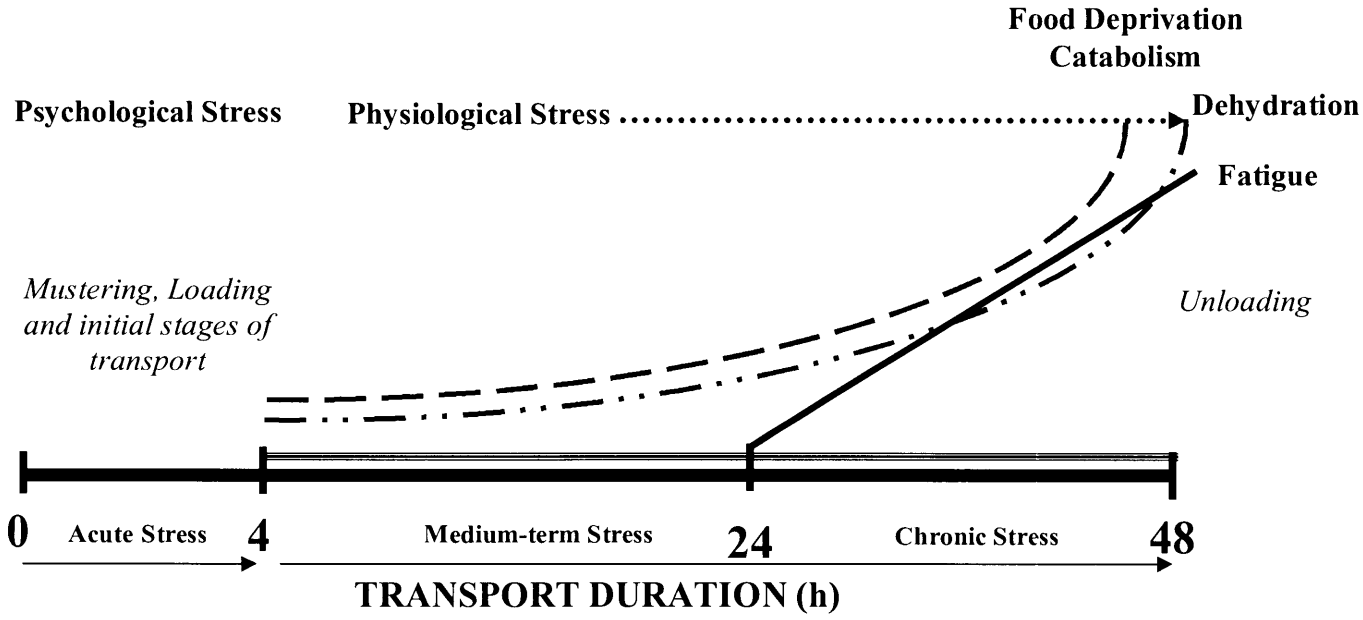


Figure 2.3 Schematic representation of different stressors that apply during long duration livestock transport

2.4 Assessment of stress in transported livestock

Behavioural, neuroendocrinal, autonomic and immunological responses to stress are closely linked (Amadori et al., 1997). A large range of biological criteria are used to assess animal welfare (Le Neindre et al., 2001). Broom (1986) identified 3 key categories for welfare assessment, namely physiology, health, and behaviour. The impact of stress on

productivity can also be considered. These four broad categories are examined further into this review.

A summary of the commonly used physiological indicators for quantifying the response to transport is listed in Table 2.4.1, which is adapted from the review of Knowles and Warriss, 2000.

Table 2.4.1 Commonly used physiological indicators of the impact of transport (from Knowles and Warriss, 2000)

Stressor	**Physiological Variable**
Food deprivation	↑ FFA, ↑ BHB, ↓ glucose, ↑urea
Dehydration	↑ Osmolality, ↑ total protein, ↑ albumin, ↑ PCV
Physical Exertion	↑ CK, ↑ lactate
Fear/arousal	↑ Cortisol, ↑ PCV
Motion sickness	↑Vasopressin
Health	↑ White cell count, Neutrophil:Lymphocyte Ratio
Fear/arousal and physical exertion	↑ Heart rate, ↑ respiration rate
Hypothermia/hyperthermia	Body temperature, skin temperature
Productivity	Weight Loss
Product Quality	Meat Quality

FFA- free fatty acids; BHB - β-hydroxybutyrate; PCV- packed-cell volume; CK- creatine kinase.

Broom (1995) emphasised that in the interpretation of physiological changes, it is important to ascertain the basal level for a measure and how it fluctuates over time. For example, plasma cortisol levels in most livestock species vary in a diurnal pattern during the day and tend to be higher during the morning than during the afternoon (Broom, 2003[b]). Therefore, critical evaluation must be applied for each physiological measure as to whether the information obtained is different from the baseline or absolute value (Broom, 2000). This requires careful analysis as it is important to be aware of the normal expected ranges for each of the physiological measures (Table 2.4.2).

Table 2.4.2 Blood analyte reference values in large animals (Source: Kaneko et al., 1997)

Analyte	Unit	Cow	Sheep	Pig
Cortisol	nmol/L	17 ±. 2	62 ±. 10	65 ±. 8
Creatine Kinase	U/L	7.4 ±. 2.4	10.3 ±. 1.6	8.9 ±. 6.0
Free Fatty Acids	mg/L	30-100	~	~
Glucose	nmol/L	3.19 ±. 0.38	3.80 ±. 0.33	6.61 ±. 0.96
Haemoglobin	g/L	110	115	130
Lactate	mmol/L	0.56-2.22	1.00-1.33	~
Total Protein	g/L	71.0 ±. 1.8	72.0 ±. 5.2	84.0 ±. 5.0
Albumin	g/L	32.9 ±. 1.3	27.0 ±. 1.9	25.9 ±. 7.1
β-Hydroxybutyrate	mmol/L	0.41 ±. 0.03	0.55 ±. 0.04	~
Urea Nitrogen	mg/dL	20-30	8-20	10-30

2.4.1 Physiological Parameters

(i) ***Cortisol***

Cortisol is a glucocorticoid that plays a major role in the regulation of glucose metabolism ensuring that enough adenosine triphosphate (ATP) is available (Tortora and Grabowski, 1993). Glucocorticoids inhibit protein synthesis, initiate proteolysis and modulate immunological intermediaries as well as mediating inflammatory reactions (Knowles and Warriss 2000). Cortisol is the most abundant glucocorticoid and is responsible for about 95% of glucocorticoid activity (Tortora and Grabowski, 1993). Transport has been shown to evoke an increase in circulating cortisol because animals will have periods of fasting, they may be psychologically challenged, especially, if undergoing transport for the first time and during this period added glucose is required by tissues to produce ATP to maintain normal body function. Any rise in circulating cortisol that is evident at different times during the transport event is interpreted as being stressful (Knowles et al., 1999; Knowles and Warriss, 2000; Mitchell et al., 1988; Tarrant and Grandin, 2000). Table 2.4.3

presents a summary of results of cortisol levels from several cattle transport studies. In general, there was a common pattern where there was an initial increase after loading and then during the next 2 to 6 hours there was a steady decline in the plasma cortisol concentrations. These results suggest that the initial acute physiological response that occurred during pre-transport handling, loading and the early stages of transport attenuated during the remainder of the journey, indicating that animals became habituated to the process of transportation.

Cortisol blood concentration is elevated in response to psychological stressors and therefore, is routinely used as an indicator of stress. However, careful interpretation of cortisol is necessary as there is potential for changes due to normal circadian effects and this may mask chronic responses. In view of this Eicher (2001) contends that it is not always an accurate measure of stress and other measures also need to be considered in the assessment of animal well-being. As emphasised by Broom (1995), understanding and identifying physiological change of such variables is important, however, to do this accurately, an understanding of the basal levels and how it may vary at different times is required.

Table 2.4.3 Summary of cortisol response to varying transport durations in cattle of different ages

Authors	Age	Transport Duration (h)	Cortisol levels at different stages of transport
Kent and Ewbank 1986[b]	1-3 week	6	Increased to maximum 5 min after loading; two to three fold pre-transport level; back to pre-transport concentration after 4 to 6 h of transport
Kent and Ewbank 1986[b]	1-3 week	18	
Kent and Ewbank, 1986[a]	3 mo	6	Increased to maximum 10 min after loading ~ 100 nmol/L; double pre-transport level
Kent and Ewbank, 1986[a]	3 mo	18	Increased to maximum 10 min after loading ~ 125 nmol/L; double pre-transport level
Kent and Ewbank, 1983	6 mo	6	Loading 96.5 ± 1.1 nmol/L
			Start transport 151.5 ± 19.8 nmol/L
			2 h into transport 230.5 ± 50.9 nmol/L
			Remaining 4 h transport 127 nmol/L
			Immediately after unloading 32 nmol/L
Warriss et al., 1995	12-18 mo	5	200% increase immediately after journey commenced from pre-transport level
Warriss et al., 1995	12-18 mo	10	88% increase immediately after journey commenced from pre-transport level
Warriss et al., 1995	12-18 mo	15	42% increase immediately after journey commenced from pre-transport level
Knowles et al., 1999	ND*	14	Increased slightly during journeys and had fully recovered to pre-transport levels 72 hour post -transport
Knowles et al., 1999	ND*	21	Increased slightly during journeys and had fully recovered to pre-transport levels 72 hour post -transport
Knowles et al., 1999	ND*	26	Increased slightly during journeys and had fully recovered to pre-transport levels 72 hour post -transport
Knowles et al., 1999	ND*	31	Increased slightly during journeys and had fully recovered to pre-transport levels 72 hour post -transport

*ND = Not defined

(ii) ***Heart rate***

Physical activity occurs during loading and unloading on and off a truck. Effort is also required to remain standing during transport. Due to this increased activity it would be expected that heart rate would be elevated. Heart rate may also become elevated as a result of a psychological stress such as handling, the presence of humans and the novelty of a new environment. Heart rate has been used in experiments as a non-invasive measure of transport related stress (Cook and Jacobson 1996; Eldridge et al., 1988; Jacobson and Cook 1998; Kenny and Tarrant 1987[ab]). It appears to be a reliable measure but only for acute or short term stressors such as those associated during handling, loading on to vehicles and certain acute effects during transport (Broom, 2000). Animals tend to habituate to transport challenges and therefore heart rate returns to or just above the basal heart rate (Eldridge et al., 1988) after a period of time during transport. In a preliminary study by Eldridge et al. (1986), the heart rates of cattle during transport were only 15% higher than that measured in cattle grazing at pasture.

From the studies of Kenny and Tarrant (1987[ab]) and Jacobson and Cook (1998), heart rate increased only at loading and unloading. However, Parrott et al. (1998[b]) reported that heart rate increased in sheep when they were loaded and remained elevated for at least 9 h of travel and then began to decline. Heart rate responses have been clearly shown to be lower if animals have had previous exposure to challenges such as transport (Broom, 2000). While an increase in physical activity will lead to an increase in heart rate, long term elevations during transport have not been observed, suggesting that the physical demands to maintain posture/balance are not high. Heart rate as a sole measure doesn't give a clear

indication of the long term impact of transport. Therefore other variables such as those that reflect other physiological states including tissue catabolism, and dehydration are required. These indicators are more useful in detecting the long term physiological impacts of transport durations.

(iii) ***Body temperature***

Sympatho-adrenal (SA) and HPA activation during transport will lead to an increase in body temperature (Swanson and Morrow-Tesch, 2001; Trunkfield and Broom, 1990). The on-board temperature, humidity, wind velocity and stocking density conditions will also have an impact on the capacity of animals to thermo regulate. Kenny and Tarrant (1987[a]) reported that an increase in rectal temperature (38.84 to 39.22 ^{o}C), although this temperature was not high it was due to close confinement on both stationary and moving trucks interfering with the heat dissipation of the cattle. However, this effect was more prevalent on stationary vehicles when there is less airflow (Fisher et al., 2004). Once the truck began to move, there was better airflow, but the improvement in airflow did not significantly reduce the increase in body temperature. Similar increases in body temperature during transport have also been observed in bulls and steers transported for durations of 4 hours (Tennessen et al., 1984). The increase in body temperature is usually in the order of 1^{o}C (Broom, 2000), but the actual value at the end of the journey will depend upon the extent of habituation and the on-board climatic conditions. Parrott et al. (1999) reported an increase in body temperature in sheep when they were loaded onto a vehicle and transported for 2.5 hours. The body temperatures increased by about 1^{o}C, and they attributed this to factors such as the exertion during loading, the muscular activity

associated with the maintenance of balance combined with the inability to dissipate heat effectively within the vehicle, and the effects of psychological stress. The pattern of temperature change in cattle has not been measured over journeys of long duration.

Body temperature may be a useful indicator of whether transport has adversely affected the health of animals. Elevated temperatures at the completion of transport and into the recovery phase could indicate an increased risk of animals exhibiting clinical signs of respiratory disease post-transport, especially in young calves (Grigor et al., 2001).

(iv) ***Creatine kinase***

Transport is a procedure that places increased physical demands on animals as it requires them to be standing for extended periods of time depending on the journey duration plus there are the demands associated with maintaining posture and balance. During transport over long durations in Australia, animals are unable to adequately rest during transport as they are not provided with space to lie down. Therefore, there is the potential for animals to become fatigued. The effort and energy required to maintain posture and balance will vary not only because of duration but it will also be dependent on the road conditions and driver expertise. This will be discussed in further detail in section 2.5.3.

Creatine kinase (CK) is an enzyme in the muscles that is released or leaks into the blood when there is muscle damage such as that occurring during muscle trauma or during vigorous exercise (Broom, 2003[b]; Broom et al., 1996). For that reason, measures of CK may be useful indicators of the physical exertion or muscle trauma associated with

transport of long duration. Measurements post transport are also useful so that recovery can be monitored, ensuring there hasn't been a long term effect of transport.

Social interactions that occur during close confinement on a truck have been observed to elicit an increase in CK (Tarrant, 1990). It would be expected that more social interactions will be exhibited if animals are loaded at a high stocking density, this is because they will have less available space to move and therefore competition for space will be greater.

Several transport studies over different durations have reported increases in blood concentrations of CK (Knowles et al., 1999; Warriss et al., 1995) as illustrated in Table 2.4.4. Swanson and Morrow-Tesch (2001) reported that plasma CK increases commensurate with journey duration. Furthermore, CK usually increases in blood especially in those cattle that stand during transport when muscle fatigue becomes a factor (Knowles et al., 1999).

Table 2.4.4 Summary of CK response to varying transport durations in cattle of different ages

Authors	Age	Transport Duration (h)	CK levels at different stages of transport
Kent and Ewbank, 1986[ab]	1-3 week old and 3 mo	6 and 18 h	Minimal change throughout different stages
Warriss et al., 1995	12-18 mo	5	Increase during transport 226 U/litre after transport, 268% increase from pre-transport level Fully recovered after 5 days
Warriss et al., 1995	12-18 mo	10	730 U/litre after transport, 855% increase from pre-transport level Fully recovered after 5 days
Warriss et al., 1995	12-18 mo	15	1039 U/litre after transport, 1698% increase from pre-transport level Fully recovered after 5 days
Knowles et al., 1999	ND*	14	Pre-transport 105 U/litre After transport 165 U/litre CK levels had declined 72 h post-transport, although had not fully recovered
Knowles et al., 1999	ND*	21	Pre-transport 105 U/litre After transport 150 U/litre CK levels had declined 72 h post-transport, although had not fully recovered
Knowles et al., 1999	ND*	26	Pre-transport 105 U/litre After transport 190 U/litre CK levels had declined 72 h post-transport, although had not fully recovered
Knowles et al., 1999	ND*	31	Pre-transport 105 U/litre After transport 140 U/litre CK levels had declined 72 h post-transport, although had not fully recovered

*ND = Not defined

In young calves which spend more time lying during transportation, an increase in CK is not generally observed (Kent and Ewbank, 1986[ab]). The difference in the results of the

Warriss et al. (1995) and the Knowles et al. (1999) studies were considerable. The animals utilised in the experiment by Warriss et al. (1995), in which cattle were trucked for durations of 5, 10 and 15 hours had a space allowance of 1 m^2 and were transported over a variety of different road types. There was no mention of lying behaviour during transport. In the duration study of Knowles et al. (1999), cattle were transported for 14, 21, 26 and 31 hours, and the animals had a space allowance of 1.55 m^2. Furthermore, the cattle were transported in pens that were well bedded with wood shavings and the transport was conducted predominantly on motorways. After a period of 24 hours 7 out of 15 animals lay down. The higher CK levels reported in the Warriss et al. (1995) study may have been due to the extra effort required to maintain posture and balance during the different road types that were travelled. The available space allowance was smaller than the Knowles et al. (1999) study and this could have also been a contributing factor due to competition for space that required increased amounts of physical interaction with other animals on the truck. Those animals that underwent the duration study up to 15 hours (Warriss et al., 1995) also had less opportunity to lay down which may have also been a causative factor for the difference in CK between the two studies.

Whilst the measure of CK is a useful indicator it is perhaps not the most informative measure of muscle fatigue. More direct measures of muscle glycogen concentration would be more useful. If animals are to be slaughtered after transport, the glycogen content is relatively easy to measure, however, *in vivo* measures require a muscle biopsy and these procedures are invasive and therefore, can potentially add to the stress imposed upon the animal.

(v) ***Dehydration***

During transport in Australia, animals do not usually have access to water. In the summer months when temperatures are high, the susceptibility of animals to dehydration is increased, particularly over long transport durations (Sinclair et al., 1992; Tarrant et al., 1992; Knowles et al., 1999). As water is an essential component for all processes which take place in the body, accounting for 60% of the total liveweight (Knowles and Warriss, 2000), avoiding dehydration is of upmost importance. The rumen can contain at least 30 to 60 litres of fluid in adult cattle and can range as high as 120 litres. During periods of water deprivation the rumen fluid can act as a buffer supplying water to the body for a period of time (Knowles and Warriss, 2000), potentially minimising the severity of dehydration.

Haemoconcentration is a useful measure of dehydration, which is evident by the changes in plasma osmolality, packed cell volume (PCV), total protein and albumin concentration (Atkinson, 1992; Fisher et al., 1999; Knowles and Warriss, 2000). The PCV is the percentage of blood occupied mainly by the red blood cells, the remainder of the volume being fluid. Therefore, as long as there is no loss or gain of cells, PCV is a useful measure of the plasma volume (Knowles and Warriss, 2000). However, as Knowles and Warriss (2000) point out stress can lead to an increase production of red blood cells through sympathetic nervous system induced splenic concentration. Therefore, other measures of dehydration such total protein and albumin need to be considered. Osmolality is perhaps the most informative measure used to assess the level of dehydration of the blood (Broom et al., 1996). Osmolality, total protein and albumin increase as the water content of the blood decreases.

Increased plasma albumin, total protein and osmolality concentrations were observed in cattle transport studies up to 31 hours in comparison with the control non-transported animals (Warriss et al., 1995; Knowles et al., 1999). These results suggest that there was a progressive degree of dehydration occurring with journey duration. Of notable importance was that adequate re-hydration of the transported animals was achieved after 2-3 days of transport with provision of food, water and rest. Carlson (1997) has reported that clinical signs of dehydration are evident when 4-6% of total liveweight of "effective" (not including fluid in the gut) total body water has been lost, moderate dehydration occurs after a 8-10% loss and severe dehydration is said to occur when liveweight losses are greater than 12%. The weight loss of animals transported 14, 21, 26 and 31 hours was no more than 7% of their initial liveweight indicating that moderate dehydration occurred. Much of the losses in liveweight that occurred during early transport were primarily from gut fill. Nevertheless, a successful recovery was achieved 2-3 days after suitable rest and adequate water consumption (Knowles et al., 1999).

(vi) ***Metabolic changes***

Cattle subjected to long distance transport will endure periods without food, which maybe up to 48 hours. During food restriction, animals will utilise their lipid reserves (carbohydrate) for energy (Knowles and Warriss, 2000). Food deprivation for periods of 12-24 hours onwards may result in decreased plasma glucose, lactate, citrate, insulin, calcium and magnesium concentrations and increased plasma free-fatty acids (FFA) and ketone (β-hydroxybutyrate - BHB), concentrations in sheep and cattle (Shorthose and Wythes, 1988).

When fat is mobilised in response to fasting, there are various changes evident in the metabolites in the blood. During fasting, the usual metabolic pathways are altered and greater amounts of ketones are produced from FFA metabolism in the liver. High levels of FFA are damaging to tissue, therefore the liver converts them to ketones such as BHB (Knowles and Warriss, 2000). Elevated levels of BHB are indicative of extended periods of fasting (Knowles et al., 1993, 1994) and are therefore a useful measure of the severity of food deprivation and the impact it is having upon the normal metabolic function of the animal. Many tissues in the body are able to use BHB better than FFA, BHB is the main energy source maintaining brain function during periods of fasting, especially in humans, however, sheep and pigs still rely on glucose (Knowles and Warriss, 2000).

Glucose, ketones and FFA are all used as energy sources to sustain metabolism during periods of food deprivation. Lengthy periods of being on a truck and attempting to maintain posture and balance can be physically demanding on cattle. During physical exertion, ketone oxidation by muscles is subsequently reduced and this results in an increase in plasma levels of FFA and BHB which have the potential to be higher than pre-transport measurements (Knowles and Warriss, 2000). Levels of these metabolites will decrease after transport as metabolism adjusts in the recovery period and following realimentation.

The blood profiles of glucose, FFA and BHB in cattle have been investigated during long distance transport for 5, 10, 14, 15, 21, 26 up to 31 hours. Glucose increased 20-41% with transport irrespective of journey time (Warriss et al., 1995; Knowles et al., 1999). This is

the reverse of what would be expected (Shorthose and Wythes, 1988). However, the increased glucose concentration could have been due to catecholamine-stimulated glycogenolysis (Warriss et al., 1995). All the cattle had recovered to the same level as the control animals two days after completion of their respective journeys. There were curvilinear increases in the concentrations of FFA for journeys of 5, 10, 14, 15, 21, 26 and 31 hours, however, there were no significant increases with increasing duration. It took 5 days for all the animals subjected to transport to recover to the control values. Blood concentrations of BHB increased for journeys of 5, 10 and 15 hours, on average by 19% in comparison with the controls, and took between 1-2 days to recover (Warriss et al., 1995). In contrast, an opposite effect was observed by Knowles et al. (1999) when cattle transported 14, 21, 26 and 31 hours. The BHB levels actually decreased in all groups during transport and were observed to increase after the journey was completed. As highlighted above, in periods of food deprivation, it was expected that BHB would increase rather than decrease. Knowles et al. (1999) suggested that this decrease may have been due to the combined effects of the journey and a diet change. The increase in BHB during recovery for both the transported and control animals was probably due to adaptation of the animals to the diet of hay after being on pasture.

(vii) ***Immunological changes***

A number of immunological changes have been observed in response to transport stress such as alterations in the total and differential white blood cell counts (Blecha et al., 1984; Schaefer et al., 1997; Eicher, 2001). These stress-induced immune system changes that occur as a result of transport (Eicher, 2001) are often referred to as immunosuppresssion.

Compounding the immunosuppressive effect is increased exposure, as a result of the marketing system, to various pathogens and feed and water deprivation (Speer et al., 2001).

Much of the work examining the immunological responses to transport has been done in calves as there has been a large focus on calf health and developing effective ways of reducing the transport stress. Kent and Ewbank, (1986[ab]) and (Murata et al., 1987) reported that calves aged 1 week to 6 months will respond to stress by increasing the numbers of circulating total white blood cells (WBC) and other specific populations of WBC (neutrophils, eosinophils). Lymphocyte numbers were decreased (lymphopenia), which along with increasing numbers of neutrophils (neutrophilia), alters the neutrophil:lymphocyte ratio (**N:L**) (Murata et al., 1987; Swanson and Morrow-Tesch, 2001), and this ratio can be predictive of disease (Amadori et al., 1997). Murata et al. (1987) also found a decrease in T-lymphocyte numbers of 4 to 6 month old calves transported 4 hours, but no change in the number of B-lymphocytes. It was found in very young calves (1-3 weeks) that changes in WBC numbers occurred approximately after 6 hours of transport (Kent and Ewbank, 1986[b]). In calves transported for longer durations of up 18 hours, WBC were back to baseline levels towards the end of the trip, however, an increase in neutrophils and a decrease in lymphocytes were also observed (Kent and Ewbank, 1986[b]).

There are few studies that report immune system measurements for older cattle subjected to transport stress (Swanson and Morrow-Tesch, 2001). Tarrant et al. (1992) used Friesian steers (n=96) and transported them for 24 hours to assess immunological changes before

and after transport. They reported that in comparison to pre-transport values, there were increases in total WBC (23%) and neutrophils (68%) and decreases in eosinophils (65%), lymphocytes (28%) and monocytes (2.6%). These data indicate that older cattle exhibit changes in their immune system to transport, but may not be as predisposed to the stress-mediated immunosuppression evident in young calves (Swanson and Morrow-Tesch, 2001). This may be because calves have been less exposed to stressful situations and are generally less adapted (Knowles and Warriss, 2000). Calves generally have not previously been mixed and have consequently, not been exposed to pathogens carried by other animals. Hence their immune systems are relatively naïve and have not been substantially challenged, which has the potential to make them more vulnerable than adult cattle.

(viii) ***Other physiological measurements***

There are several other neuroendocrinal measures that have been applied in animal stress studies e.g. prolactin and adrenaline. The secretion of prolactin occurs when an animal has been exposed to stress (Moberg, 2000). Prolactin was elevated at loading and the early stages of transport in a study investigating the hormonal and physiological effects of a 15 hour road journey with sheep (Broom et al., 1996). The long term effects of transport on prolactin release were found to be small.

Adrenaline and noradrenaline are also released when animals have been subjected to stress (Matteri et al., 2000). Changes in these adrenal medullary hormones occur rapidly and their measurement has not been utilised much during the assessment of welfare during

transport (Broom, 2003[b]). Neither adrenaline or noradrenaline are likely to give an indication of the impact of transport of long duration on the well-being of animals.

Detection of acute phase proteins is useful following tissue trauma and swelling (Murata and Miyamoto, 1993). The acute phase protein haptoglobin maybe potentially useful in determining the impact of transport on the immune system (Murata and Miyamoto, 1993). Bovine haptoglobin is produced following tissue injury and inflammation (Murata and Miyamoto, 1993). One of the early physiological responses to disease and inflammation is the pro-inflammatory response which involves a complex set of reactions involving the release of multiple soluble mediators, which impact on the host's metabolic response to inflammation (Arthington et al., 1993). An important group of soluble mediators include the acute phase proteins and in response to stress stimuli, blood concentrations of these acute phase proteins increase in cattle (Conner et al., 1988).

Increases in haptoglobin have been noted after transport in 6 month old calves trucked 1400 km over two days to a feedlot, including being fed and watered at 3 stops during the journey (Murata and Miyamoto, 1993). From this study there were indications that transport was having an impact on homeostatic mechanisms that can influence subsequent immune responses (Eicher, 2001). Haptoglobin levels can remain elevated for up to 4 days (Peppard et al., 1994) after transport and based on these findings it is apparent that both innate and adaptive immunity are affected by transport (Eicher, 2001). Therefore haptoglobin has merits as an indicator of any sustained effect of transport stress.

2.4.2 Behaviour

Changes in behaviour are indicators that an animal is having difficultly adjusting to a situation and these signs may be useful for assessing animal welfare (Broom, 2000). If cattle are observed closely at the beginning of a journey they are generally anxious and tend to exhibit signs of restlessness (Knowles, 1999; Swanson and Morrow-Tesch, 2001). Tarrant (1990) documented that this restlessness was manifested by the frequency of changing position in the vehicle and the increase in urination and defecation. Animals have a tendency to defecate and urinate more frequently when stressed (Tarrant, 1990). Similarly, Knowles (1999) reported that the incidence of social interactions increased when animals were stressed during social regrouping and confinement on a truck. The incidence of interactions was initially high but then gradually decreased as the hierarchical structure was sorted amongst the group. The actual stocking densities used in this experiment was not documented but this will undoubtedly have an affect on the expression of these behaviours. Investigative behaviour tends to decrease after the first 4 hours of transport and few or no aggressive or sexual behaviours are generally exhibited (Tarrant et al., 1992). In the study by Tarrant et al. (1992), this pattern of exploratory and aggressive behaviour was similar at low (1.33-1.41 m^2/head), medium (1.19-1.24 m^2/head) and high (1.03-1.08 m^2/head) stocking densities.

Cattle prefer to stand during transport as lying down can produce considerable pressure on those parts of the body in contact with the floor of the truck (Knowles and Warriss, 2000). However, Tarrant et al. (1992) found that steers being transported at a stocking density of 1.03 m^2/head began to lie down after 16 hours of transport. Likewise in a study by

Knowles et al. (1999), cattle with a mean liveweight of 570 kg started to lie down after 24 hours of transport. From the study of Knowles et al. (1999), there were no differences in the CK levels of the animals lying compared to those that remained standing, however, differences were apparent in plasma cortisol concentrations. The animals that lay down had higher cortisol concentrations than those that remained standing. Knowles et al (1999) reasoned that this was because of the animals having to maintain balance while in the lying position, and this task became physically taxing towards the end of the journey, compounded by other animals potentially falling on them during the journey. Therefore, these results suggest that if cattle are given enough space to lie down they will do so after 16 – 24 hours of transport.

A study by Tadich et al. (2000) reported that 36 hours of transport without a rest period was detrimental to the welfare of cattle due to the increased levels of cortisol, glucose and CK in the blood. The authors have not stated whether lying behaviour was observed during transport. The increase in blood creatine kinase and decrease in muscle glycogen over journeys of up to 31 hours provides some indication of the physical effort required to remain standing and maintain balance against the motion of the truck (Knowles et al., 1999). Whilst there was a gradual decrease in the glycogen content of muscles, the levels were always comparable with those observed in unfatigued animals (Knowles et al., 1999).

While cattle do prefer to stand during transport, what is not well understood is the effects of fatigue over longer journey durations greater than 24 hours. Research is required in this

area to determine if there is a detrimental impact upon animals if they are unable to lie during transport over long durations.

From studies by Kenny and Tarrant, (1987[ab]); Eldridge et al. (1988); Lambooy and Hulsegge (1988), the most common standing orientation for cattle on trucks appears to be either perpendicular or parallel to the direction of motion, as this enables cattle to maintain better balance.

In general, the data on behaviour during transport is limited. If this can be obtained it will provide information about the activities of the animals (Tarrant, 1990) and how they are coping with the transport environment. It identifies whether they are standing, lying, falling, slipping or being trampled, and what may be the potential causes of this. The collection of behavioural data may assist in identifying where improvements can be made to improve transport conditions.

2.4.3 Productivity, health and product quality

(i) ***Weight loss***

When livestock undergo transport of any duration, they will endure a period without food and water, which will result in losses in liveweight (Knowles, 1999). The rate of liveweight loss is greatest during the first 12 hours of food and water deprivation and slows down thereafter as summarised by Wythes (1987), (Table 2.4.5).

Table 2.4.5 Weight loss for cattle without feed and water (Wythes, 1987)

Hours without feed and water	Liveweight Loss	
	kg	%
6	10	2.5
12	16	4.0
24	24	6.0
48	40	10.0
72	48	12.0

The source of the weight loss varies over a period of food and water deprivation. During the initial stages, the majority of weight loss comes from faeces and urine. In a study by Phillips et al. (1991), the combined weight of urine and faeces excreted accounted for 61 – 64% of the total liveweight loss after 48 hours of food and water deprivation. During prolonged periods of fasting which extend beyond 48 hours, tissue catabolism and dehydration increase in their contribution to liveweight loss. Studies by Galyean et al. (1981) and Phillips et al. (1991) found no significant differences between the weight lost by fasted animals compared with animals that had been fasted and transported over durations of 32 and 48 hours, respectively. It is important to note however, that these cattle were not trucked continuously for 32 or 48 hours, it included both fasting and transport.

Wythes and Shorthose (1984) reported that carcase weight loss occurs through dehydration of carcase tissues and mobilisation of depot fat and muscle glycogen and this was generally not observed until after 24 hours of food and water deprivation in cattle. Previous feeding level, in particular the amount of roughage livestock have consumed, will influence the level of weight loss during transport (Warriss, 1990). Roughage prior to transport will

potentially reduce the amount of weight loss as it has the tendency to slow down the rate of passage through the gastrointestinal tract (K.Sullivan 2002, pers. comm.).

In cattle transport studies by Warriss et al. (1995) and Knowles et al. (1999) where the effects of transport duration for 5 - 31 h were investigated, all the weight loss that occurred during transport had returned 3-5 days after the journey had been completed when the cattle had access to good quality feed and water.

(ii) ***Meat quality***

The impact of transport on carcase and meat quality has been mainly assessed via the incidence of bruising (Knowles, 1999) and dark cutting at slaughter. As discussed earlier in this review, transport can be physically and psychologically demanding on animals which can result in glycogenolysis in muscles. This in turn can have significant negative effects for meat quality depending on the magnitude of the loss in muscle glycogen. Glycogen is an energy source and is stored in muscle cells. After death, the glycogen reserves in the muscle are converted to lactate in a process called glycolysis. As a consequence, the pH of muscles fall from about 7 (at death) to values of around 5.4 to 5.6 (Gardner and Pethick, 2004; Tarrant and Grandin, 2000). If pre-slaughter muscle glycogen levels fall below a critical threshold of 45-55 μmol/g, ultimate pH will be above the normal range of 5.4-5.6 (Warriss, 1990). The condition known as "dark cutting" occurs at pH $\geq$ 5.7 and such meat is discounted in the market place. Apart from its characteristic dark colour and dry appearance, high ultimate pH meat (especially at pH 5.9 – 6.2) is generally tough as well (Gregory, 1998; Gardner and Pethick, 2004; Tarrant and Grandin, 2000).

Cattle that are destined for slaughter are placed in lairage after transport. Shorthose and Wythes (1988) reported that adequate rest alone in lairage will allow cattle to recover and restore muscle glycogen concentrations to levels which are sufficient to ensure low ultimate pH at slaughter. For this to occur, handling of animals during this time should be performed with care and weather conditions need to be favourable. The appropriate length of time that cattle are rested depends upon the distance that they have travelled (Shorthose and Wythes, 1988). After short journeys ($\leq$6 hours) they are normally slaughtered the next day, however, after journeys of significantly longer duration they may be left to rest for one and sometimes two days, to recover (Wythes and Shorthose, 1984). Warriss (1990) stated that the recovery of glycogen levels after they have been depleted appears to take at least two days without any disturbance and with access to feed and water to adequately recover. This is supported by Tarrant (1988) who reported that feed intake during recovery was essential, as fasting almost eliminates glycogen resynthesis in beef muscle. To summarise these results, whilst rest in lairage is essential, the additional provision of feed would guarantee glycogen repletion to satisfactory levels at slaughter. Feeding a high energy diet during recovery not only accelerates glycogen repletion rates but also leads to higher muscle glycogen concentrations (Tarrant, 1998). Where possible, providing feed of high energy prior to transport and in the recovery period would expedite glycogen replenishment and ensure that satisfactory levels of glycogen are achieved prior to slaughter, thus reducing the incidence of dark cutting in beef meat.

Bruising occurs most often on the muscles around the pinbone, hipbone and shoulder regions (Honkavaara et al., 2003). Reports of carcase bruising as a result of transport are

clearly an important indicator of substantial problems for animals from an animal welfare standpoint (Broom, 2000). Carcase bruising may occur prior to transport due to poor handling at loading and this can be compounded by attempting to load animals in poorly designed facilities. During transport, animals may be susceptible to bruising as a result of the stocking density. Eldridge et al. (1988) reported that the space allowance for animals during transport can significantly influence carcase weight, bruising and subsequently the welfare of the animals. This is primarily due to close confinement and an increase in the amount of jostling that occurs in order for animals to retain the space they need to maintain their footing without falling over and subsequently being trampled. The incidence of carcase bruising can also increase due to unsatisfactory road conditions and driving style (Tarrant, 1990). It is also important to note that McNally and Warriss (1996) reported that bruising was increased with distance travelled. Similarly, Shorthose and Wythes (1984) reported that the incidence of bruising during transport appears to increase with distance travelled. Bruising increased as distance travelled increased from 50 to 1200 km and decreased as the distance travelled increased further from 1200 to 2000 km, however, there were large variations in this study.

Meat quality is generally only affected in extreme situations during transport when transport conditions are not optimal. Moreover, improper handling at loading and unloading and poor driving can cause bruising, injuries and a reduction in meat quality (Honkavaara et al., 2003). From a study in Victoria where the bruising at two abattoirs was examined on 271 cattle, McCausland and Millar (1982) estimated that 10-20% of bruising

occurred before or during transport and the remainder of the bruising may have occurred at the abattoir.

(iii) ***Health***

Young calves are especially vulnerable to transport, more so than mature cattle due to their naïve immune systems and lack of exposure to new environments (Swanson and Morrow-Tesch, 2001). Transport mortality in mature beef cattle is generally very low (Knowles, 1999). Calf mortality during transport also tends to be low; however, mortality rates following transport can be high and this is usually because of disease (Knowles and Warriss, 2000). Bovine Respiratory Disease (BRD) and diarrhoea are significant health issues in young newly weaned transported animals and these contribute to calf morbidity and mortality (Swanson and Morrow-Tesch, 2001)

The duration of transport in younger animals should be kept to a minimum where possible. Mormede et al. (1982) found less post-transport disease amongst young cattle whose transport and marketing took only 13 hours compared with 37 hours.

2.5 Factors that affect the welfare of cattle during livestock transport

The severity of transportation stress depends on several factors. These include the class of stock, their physical condition, pregnancy status, pre-transport preparation (e.g. handling, pre-transport nutrition and feed and water deprivation) and transport conditions (Eicher, 2001; Hartung et al., 2003; Kent and Ewbank, 1983; Knowles, 1999; Trunkfield and Broom, 1990). The latter includes many factors such as on-board environmental

conditions, air flow and quality, along with stock crate design. Location in the vehicle, driving standards and road surface conditions are all important factors (Fazio and Ferlazzo, 2003), combined with stocking density, duration of transport, and feed and water deprivation (Dalin et al., 1993). These factors are discussed in further detail below.

2.5.1 Stock class and condition

Age, sex and physiological condition affect the response of animals to handling and transport (Fazio and Ferlazzo, 2003). There are some classes of stock that are particularly at risk during transport, including young calves and pregnant cows. Each of these classes has special nutritional needs that must be taken into account when planning their transport (Matthews et al., 2000). The age of cattle may affect the ability of animals to cope with transportation stress (Matthews et al., 2000). For example, care should be taken when trucking young stock (calves 4-15 days of age) as they are less stable on their feet and may tire easily as a result of the stressors of handling and transport.

In a sequence of studies, Kent and Ewbank (1983, 1986[ab]) also provide evidence that age may influence the magnitude of the physiological response to transport. They did however report that 1-3 week old calves were least affected, followed by 3-4 month old calves, with 6-month-old calves being the most affected by transport because of their raised plasma corticosteroid concentration. This evidence affirms the results of Mormède et al. (1982) who suggests that younger calves may show less response to transport due to the incomplete development of the HPA axis.

(i) ***Pregnancy status***

Transport of pregnant female cattle during the final month of pregnancy is prohibited in Australia (SCARM, 2002). One reason for the greater care required when transporting pregnant females is their altered metabolic state, causing them to be less tolerant of stressful conditions such as transport and feed restrictions (Toharmat and Kume, 1996). The stress of transport and feed deprivation combine to negatively affect the ability of pregnant animals to maintain normal carbohydrate metabolism (Shorthose and Wythes, 1988). This is a cause for concern as cattle are deprived of food and water during transport and this may subsequently increase the risk of metabolic diseases such as ketosis in pregnant cattle (Radostits et al., 1994). Work by Fisher et al. (1999) in New Zealand illustrated the effect of long haul transport on pregnant, non-lactating dairy cows with cow liveweights declining by 6-9% during the transportation process. They concluded that transported pregnant cows benefited from overnight rest, feeding and watering to ensure hydration and some replenishment of muscle glycogen. However, it was noted that liveweight and serum magnesium concentrations were significantly reduced by the overall journey, emphasising the requirement for suitable mineral supplementation and careful feeding of pregnant cows before long haul transport.

(ii) ***Physical condition***

The metabolic state and physical condition of an animal is a critical factor in determining its ability to cope with a major stressor such as transport. Animals that are in poor condition and generally weak may pose a welfare risk. The land transport code of practice for cattle (SCARM, 2002) specifies that cattle should not become so weak that they are not

fit to travel and where transportation is necessary, weak cattle should be trucked to their destination as quickly as possible and they should be protected from extremes of weather and transported with stock of similar condition. Pre-conditioning feeding treatments can be an important strategy to reduce transport stress in cattle (Schaefer et al., 1997) which is discussed in more detail below.

2.5.2 Pre transport handling and preparation

(i) ***Pre – transport handling***

It is imperative that best possible handling practices are utilised during preparation and loading for transport, thus minimising the likelihood of poor welfare occurring at this point (Tarrant, 1990). Animal welfare during loading can also be further improved by utilising well designed facilities based upon an understanding of the animal's natural behaviour that facilitate low stress handling (Grandin, 1993).

Careful quiet handling is therefore important in order to keep fear to a minimum (Grandin, 1997). The response to fear will vary between animals due to genetic factors (e.g. temperament) and previous experience. These factors interact in a complex way to determine how fearful an animal will become when it is handled and transported (Grandin, 1997).

(ii) ***Loading***

Loading and the early stages of transport have been shown to be psychologically and physically stressful for cattle (Agnes, et al., 1990; Tennessen et. al., 1984; Kent and

Ewbank, 1983, 1986[ab]; Trunkfield and Broom, 1990) and sheep (Broom et al., 1996; Parrott et al., 1998 [ab]). In these studies, increases in the physiological indicators of stress (cortisol, heart rate and respiration rate) have been observed immediately after loading, but then decreased during transport. The magnitude of changes of cortisol concentrations were 2 to 3 fold in 1-3 week old calves (Kent and Ewbank, 1986[b]) and has high as eleven fold increase in six month old calves (Kent and Ewbank, 1983) at loading and in the early stages of transport. However, the majority of the variables were close to pre-transport values at the conclusion of their journeys. Careful, quiet handling in well designed facilities will assist in minimising the impact of the psychological and physiological stress imposed upon animals at loading.

(iii) ***Mixing of animals***

Prior to transport, animals are sometimes mixed with unfamiliar animals and this can present a major social stressor. Mixing will often result in increased levels of antagonistic behaviour, including fighting, which may lead to injury and in severe cases, death (Tarrant, 1990). The level of such behaviours may not be high during transport because of the reduction in available space (Tarrant, 1990).

A study undertaken to examine the impact of mixing unfamiliar groups of feedlot cattle 1, 2 and 4 weeks before slaughter showed little effect of mixing on the physiological variables associated with physiological responses, however there were noticeable effects on meat quality of the group mixed 1 week prior to slaughter (Colditz et al., in press). They reported that mixing of feedlot cattle less than 2 weeks before slaughter may compromise meat

quality. Therefore, mixing groups of unfamiliar cattle should be avoided where possible within 2 weeks of transport, especially if the animals that are going to be transported are destined for slaughter. Whilst the study did not reveal any major differences in physiological stress indicators, care should be taken when mixing animals prior to transport, ultimately to minimise any potential impact this may have on animal welfare.

Horned and polled cattle should not be transported together. As expected, horned cattle experience more bruising in transit than polled or tipped cattle (Ramsay et al., 1976). This was supported by the research of Wythes et al. (1985) where they investigated the level of bruising and found significant differences between slaughter-lots due to horn status. A mixed horn group of animals was significantly more bruised than a polled group.

(iv) ***Pre-transport feed and water deprivation***

In preparation for transport, it is common for cattle to undergo some period of food and water deprivation prior to transport. This period is often referred to as "curfew" within the Australian cattle industry. The length of the curfew periods can vary considerably but is generally around 6-12 h. The main objective of a curfew period is to minimise the amount of faeces and urine that is excreted once the animals are loaded on the truck, thus minimising slippage, and reducing hide contamination from animal excreta. There is a perception in the Australian livestock transport industry that cattle that have been subjected to a curfew tend to travel better. There is some anecdotal industry evidence to support this perception, but there is little scientific evidence to confirm whether curfews are beneficial or not.

The benefits of a pre-transport period of feed and water deprivation, upon animal welfare, during transport are unclear as there is very little published data. In one Irish study Earley et al. (2004) found no significant difference in the live weights between 8-month old bulls fasted and not fasted for 8 hours followed by 8 hours of transport. There was certainly no impact upon animal welfare evident from their study. Similarly, Jacobson and Cook (1997) reported that fasting bulls for a period of 20 hours before transport for 2 hours did not increase the physiological response to transport when compared to non fasted bulls however, fasting was detrimental to carcase weight.

(v) ***Pre-transport conditioning strategies***

Pre-transport feeding treatments have been examined in the context of alleviating the effects of pre-transport stress. Most of the research has focused on the use of electrolytes and carbohydrate therapies. These include the use of vitamin treatments and the feeding of fats in the diet, both of which have had varying degrees of success (Cole et al., 1982) with regard to improving the rates of recovery of transported animals. Most attention however, has been focused on the use of electrolytes and high energy supplements (Schaefer et al., 1997). Several experiments have been performed investigating the effects of adding electrolytes and carbohydrates to drinking water prior to or during livestock transport to reduce the impact of dehydration (Knowles et al., 1997; Schaefer et al., 1990, 1997).

It is believed that the physiological demands of animals prior to and during transport are similar to the increased workload placed on an athlete's body during vigorous exercise (Schaefer et al., 1997). There have been positive results in humans after consuming

electrolytes which are fluid supplements that help control fluid levels in the body during periods of increased physical activity. Specifically, there were reductions in states such as dehydration, fatigue and muscle catabolism (Schaefer et al., 1997). On this basis, Schaefer et al. (1997) provided oral electrolytes to cattle pre-transport which was found to minimise reductions in both live and carcase weight loss during transport, as well as reductions in the incidence of dark cutting.

The addition of electrolytes to drinking water during the post -transport recovery phase has also been shown to decrease the rate of carcase weight loss over a 36 hour period. Animals that consumed electrolytes lost 0.31kg/h, as opposed to 0.71kg/h in the non-electrolyte supplemented group (Jones et al., 1992). Electrolyte supplementation was also found to be beneficial, particularly in young calves, when administered during or after transport (Knowles et al., 1999). The major benefits of the addition of electrolytes appear to be in reducing dehydration and initiating an earlier return to eating. However, in a study by Burrow et al. (1998), where the effect of electrolyte supplementation on weight loss of steers and heifers was evaluated during long distance transport (1500 km), there were no advantages observed. A mitigating factor here was that molasses and cottonseed meal were fed to the animals prior to trucking and this may have provided sufficient energy that may have negated any additional benefit of electrolytes (Colditz et al., 2006). This finding of a lack of any real benefits through pre-transport supplementation of electrolyte solutions was corroborated in a subsequent study by Parker et al. (2003). For *Bos indicus* cattle transported for 48 hours they observed no obvious benefits of utilising electrolyte solutions over that of providing water prior to transport.

2.5.3 Transport conditions

(i) ***Environmental conditions during transport***

Temperature, humidity and air flow are the major environmental factors that have the potential to affect animal welfare during transport. The temperature and stress experienced by animals during transport will depend upon a combination of factors including ambient conditions, whether the truck is stationary or moving, stocking density, location on the truck and the class and condition of the stock being transported (Matthews et al., 2000). Heat stress is generally a result of a combination of high temperature and humidity (Randall, 1993; Silanikove, 2000). At low humidity levels, cattle can be very tolerant of warm conditions (Armstrong, 1994), but increasing humidity severely limits the effectiveness of body cooling mechanisms (Armstrong, 1994), particularly in *Bos taurus* breeds.

The ambient air temperature is one of the major factors influencing temperature inside stock crates (Matthews et al., 2000), and typically temperature within the truck increases during loading; decreases when the truck begins to move; remains relatively constant during the journey and increases when the vehicle stops before unloading the stock (Knowles et al., 1996).

Whilst temperature alone is important as previously stated, it is the combination of temperature and relative humidity that is most critical to the animal because high humidity reduces the effectiveness of thermoregulatory mechanisms. These two variables can be captured in a single temperature and humidity index (THI) and the THI is commonly used

to assess livestock environments particularly in the context of heat stress (Armstrong 1994; Silanikove, 2000). The THI is derived using the combination of wet (W) and dry (D) bulb temperatures using the formula (Silanikove, 2000).

$$THI = 0.72(W^{\circ}C + D^{\circ}C) + 40.6$$

Values for the THI between 75-80 represent mild thermal stress, while values $\geq$80 indicate moderate to severe heat stress (Silanikove, 2000).

Work by Wathes et al. (1983) in the United Kingdom reported that cattle are able to acclimatise to temperatures up to 27°C and calves up to 30°C, and that both cattle and calves are very tolerant of cold conditions. Likewise, Knowles (1999) recommended that temperatures of 30°C should not be exceeded in livestock trucks. If this is the case, then the main concern during transport is to maintain temperatures inside and outside the stock crate in the vicinity of 27-30°C. Generally, for temperatures below 30°C, humidity is of little consequence but transport near this temperature combined with high humidity should be avoided (Knowles, 1999) in the United Kingdom.

Climatic conditions throughout Australia are very different to that experienced in the United Kingdom and cattle are regularly exposed to summer temperatures of greater than 30°C, often in combination with high humidity. Some Australian cattle are able to cope with temperatures in excess of 30°C especially *Bos indicus* derived cattle breeds that occupy a large proportion of northern Australia. These cattle have the ability to adapt to high heat and high relative humidity (RH) better than the *Bos taurus* breeds (Newman and Coffey, 1999). However, it is important to remember that sudden changes in temperature

can cause acute stress (Matthews et al., 2000), and this needs to be considered when planning movement of cattle in the hotter months throughout Australia, particularly when moving from temperate to sub-tropical or tropical regions.

There are no known reports of measurements of temperature and humidity on cattle trucks travelling within Australia; although it has been reported from research performed in the United Kingdom that during long journeys, temperature in particular is likely to vary considerably (Warriss et al., 1995; Knowles et al., 1999).

Studies of the physiological responses in *Bos taurus* and *Bos indicus* cattle to prolonged, continuous heat and humidity have however, been undertaken (Beatty et al., 2006). Both breed types were exposed in climate controlled rooms for four days of gradually increasing temperature, followed by a hot period of 5 days at 32°C wet bulb temperature or above. The conclusions from this work were that both breed types had the ability to maintain blood gas homeostasis (partial pressure of carbon dioxide and oxygen and the bicarbonate levels in venous blood) during periods of high heat and humidity. *Bos taurus* cattle did experience physiological changes such as open mouth panting, drooling, reluctance or inability to rise and increased licking of their coat. They were more affected by continuous and prolonged high heat and humidity than the *Bos indicus* cattle, which experienced similar, but less pronounced changes. Whilst open mouth panting was observed in *Bos taurus* cattle, there was no evidence of prolonged heat stress in either breed type.

Depending on prior adaptation, these results confirm that *Bos indicus* cattle can cope better with high temperatures and RH compared with *Bos taurus* cattle (Gaughan et al., 1999; Newman and Coffey, 1999) and this needs to be considered when planning a journey during extreme hot weather.

The impact of cold, wet and windy weather conditions during transport has not been investigated in Australia. Whilst parts of Southern Australia can be cold, it is still well above the extreme cold temperatures of other parts of the world (e.g. Europe). However, these conditions may still be a potential risk to animals that are in poor condition. In general, cold weather does not pose the same risk to adult cattle that hot weather does in Australia, with the possible exception of young calves who are relatively cold sensitive at birth (Hemsworth et al., 1995).

(ii) ***Air flow quality and stock crate design***

Air quality on livestock transport vehicles is a function of both the incoming air and the immediate environment within the vehicle. The incoming air will affect air quality inside the truck by regulating temperature, relative humidity, gas levels and other contaminants (Wikner et al., 2003). Air quality can be contaminated by exhaust fumes and dust from the vehicle. The latter is significant when travelling on dirt roads and the increased grit can potentially enter the animal's airways and eyes causing irritation.

Other contaminants affecting air quality include ammonia, carbon dioxide and hydrogen sulphide (Wikner et al., 2003). Carbon dioxide is exhaled from animals at high

concentrations (Wathes et al., 1983), whilst hydrogen sulphide and ammonia are released from faeces and urine (Matthews et al., 2000; Wikner et al., 2003).

The design features of the livestock crate will have a major influence on the degree of comfort experienced by animals during transport. A major design consideration is the size and positioning of ventilation slots on the stock crates with the objective being to maintain adequate ventilation to the animals being transported (Matthews et al., 2000). The range in animal size must be taken into account when designing the shape and positioning of the ventilation slots (Matthews et al., 2000). For example, on a cattle crate they need to be positioned high enough so that they can not be blocked by cattle thus reducing airflow. On a moving truck, ventilation occurs through side vents due to forced air motion arising from pressure differences between inside and outside the vehicle (Randall, 1993). When a truck is stationary, ventilation is provided only by natural convection (Randall, 1993) and this could cause deterioration of the on-board environmental conditions especially in summer. This effect can be further compounded by close confinement of animals resulting in above average temperatures because of reduced airflow. Under these conditions heat stress can occur (Matthews et al., 2000).

(iii) ***Location in the stock crate***

Environmental conditions will vary in each compartment of the stock crate, for example animals standing in the middle of the pens of the crate are likely to experience higher temperatures and less air flow than those animals that are standing on the outer edge of the pens near the ventilation slots (Matthews et al., 2000). This temperature variation will be

more prevalent on sheep trucks as they are usually loaded at higher stocking densities compared with cattle trucks. This is supported by Jarvis and Cockram (1999) who investigated the environmental conditions on sheep trucks in the United Kingdom. This study was undertaken in the cooler months using a three-deck livestock truck. The highest temperatures observed were in the midsection of the middle deck in both stationary and moving vehicles. On the moving vehicle the differences were in the order of 0.9°C. However, Warriss et al. (1995) reported no differences between temperatures recorded in the front, middle or rear pens of a truck carrying 24 steers. If temperatures had been monitored while the vehicle was stationary, it is possible more variation may have been observed. Fisher et al. (2002) investigated during summer in New Zealand the conditions for lambs on road transport vehicles, combined with inter-island ferry transport. The conclusion from this study was that if stocking densities were lowered during summer, it eliminated potential problems of lambs suffering or dying on hot days. Fisher et al. (2002, 2004) reported that THI increased when the vehicle was stationary, it should be noted that in these experiments measurements were recorded on the bottom and middle decks of three and four deck trailers. There are no reported measurements of on board temperatures in Australia for either sheep or cattle.

(iv) ***Road conditions and driving quality***

In Australia, the road conditions during livestock transport can vary considerably both in terms of road surfaces and types. In a study by Eldridge et al. (1988), it was reported that the heart rates of beef heifers were higher when travelling on rough country roads or suburban roads with frequent intersections, compared with travelling along smooth

highways. Matthews et al. (2000) suggested that this could be partly due to increased physical activity that was necessary to maintain footing on the rougher roads. Bradshaw et al. (1996) conducted a study with pigs and sheep that were transported on a rough and a smooth road. The rough roads were narrow and the smooth roads were highways. The conclusions from their study were that salivary cortisol concentrations were highest in animals that undertook the rough road, 14.6 nmol/L (pigs) and 11.13 nmol/L (sheep), compared with 8.5 nmol/L (pigs) and 5.5 nmol/L (sheep) over the smooth roads. Bad road conditions have the potential to fatigue animals quickly as well as impairing their health and welfare (Hartung, 2003).

The accumulation of faeces and urine on the floor of the stock crate can also increase the risk of slippages, particularly on rough and/or winding roads. Vibration can also be of concern as it will potentially influence postural instability. Continuous vibration is likely to affect the level of discomfort experienced by animals and may even result in muscular fatigue (Cockram et al., 2004). The level of vibration is also likely to be affected if the road conditions are poor.

The quality of the driving can have a major influence on animal welfare during transport as poor driving can lead to increased risk of injury and it affects the ability of the animals to habituate to the transport event. Driving style and driving events can directly affect cattle during transport (Kenny and Tarrant, 1987[ab]; Tarrant et al., 1988; Tarrant et al., 1992; Cockram et al., 2004). Specifically braking, gear changes and hard cornering account for the majority of losses of balance (Tarrant et al., 1988; Kenny and Tarrant, 1987[ab]). Table

2.5.1 illustrates the effect of specific driving events on the incidence of loss of balance and falls during transport of cattle.

Table 2.5.1 The percentage of balance problems accounted for by specific driving events during 4 h road transport of Friesian steers (Source: Tarrant et al., 1988)

Driving Events	Loss of Balance	Struggle for footing	Goes down momentarily	Goes down and stays down
Gear Changes	9	11	14	0
Cornering	16	29	36	33
Bumping	4	2	2	17
Two events coinciding	12	6	2	0
Braking	11	6	2	0
Starting or Stopping	7	2	0	0
TOTAL	59%	56%	56%	50%

Of the losses of balance, the majority occurred during specific driving events, namely braking and cornering. These specific driving events also accounted for 90% of the losses in balance in the Kenny and Tarrant (1987[ab]) studies where steers and young bulls were transported for 1 hour. If animals fall during transport as a result of breaking and cornering, this may result in bruising, animals being trampled and unable to get back on their feet, which in turn, may also lead to destabilisation of other animals in the load.

(v) ***Stocking density***

The land transport code of practice for cattle (SCARM, 2002) details the recommended average stocking densities for cattle of various liveweights which includes available floor area per head and the number of cattle that should be loaded per deck. However, in

different Australian states there are additional regulations which also apply. In some states, for example Queensland, loading is by volume which means that the truck can be loaded at the optimum rate whilst in other states they have a weight restriction that must be complied with and this may mean the truck is not fully loaded.

In general, there is relatively little information on the appropriate dimensions for animals of different sizes and their needs during transport of different durations and how transport vehicle design interacts with issues like stocking rates to optimise animal welfare (Hartung et al., 2003). Stocking density clearly affects livestock during transport (Matthews et al., 2000) as highlighted in the study by Eldridge et al. (1988). This study indicated that animals that were transported in small pens were less affected by variation in space allowance than those animals transported in large pens as determined by the changes in heart rate and animal movement. Eldridge et al. (1988) reported that the number of movements was six times greater in large pens with low stocking density (high space allowance) when compared with small pens with high stocking density (low space allowance). It is also reasonable to extrapolate from these results that at high space allowances there is an increased risk of losses of balance due to the lack of mutual support in response to rapid changes in vehicle motion, particularly in large pens.

High and low stocking densities can be detrimental to cattle welfare in different ways (Matthews et al., 2000). Cattle require space in which to make positional adjustments during transport, and at high densities, they are not able to move freely which can lead to difficulty in maintaining balance (Tarrant et al., 1988). When animals are unable to regain

their footing during a loss of balance they go down, become trapped on the floor and may be seriously injured. Tarrant et al. (1992) showed that for cattle, this risk was greatly increased at high stocking densities. However, if cattle are loaded at low stocking densities they have an increased chance of slipping and/or falling due to too much available space.

In the study by Tarrant et al. (1998), Friesian steers that weighed between 500 to 735 kg were transported for 4 hours. The following stocking densities were used, low (3.27 m^2/animal), medium (1.96 m^2/animal) and high (1.09 m^2/animal). The results of this study indicated that plasma cortisol and carcase bruising increased with stocking density.

These results were supported by the findings of Knowles (1999), in which there were elevated levels of creatine kinase in the plasma, reflecting muscle damage in steers that were trucked at higher densities. Severe bruising occurred only at the highest stocking density. Further confirmation of these results was found in a study by Eldridge and Winfield (1998) where Hereford steers (400 kg liveweight) were transported for 6 hours at high (0.89 m^2/animal), medium (1.16 m^2/animal) or low (1.39 m^2/animal) stocking densities. Animals in the low and high stocking densities had the highest bruise scores at slaughter. Medium stocking densities should be utilised where possible to minimise potential bruising.

The space available to livestock during transport is an important consideration as it can also indirectly affect the environmental conditions on the truck while also affecting livestock welfare (Matthews et al., 2000). Knowles (1999) reviewed road transport practices for

cattle and suggested that there appears to be an optimal stocking density that has not yet been clearly established for the majority of transport conditions. The review provides guidelines for various countries throughout the EU and in so doing, probably encompasses the optimum stocking density for many types of animals. However "ideal" stocking densities have not been clearly documented because factors such as duration, on-board conditions, age and size of the animals also need to be considered.

Groups of cattle may contain individuals that are variable in size, compared to pigs and sheep, where most are relatively similar in size and weight within a group. The total space requirements of a group of cattle that are all of similar size and weight will possibly be different from those of a more variable group and this should be considered when transportation by road is necessary.

In contrasting the recommended space allowances in Australia and the EU, there are obvious differences. It is important to note that in the EU recommended space allowances are different for journeys of short (<8 hours) and long durations (>8 hours) (Commission of the European Communities, 2002). For a 250 kg animal undertaking a journey <8 hours, the recommendation is 0.85 m^2/animal and this is increased to 0.93 m^2/animal for journeys > 8 hours. An additional 10% is recommended for pregnant females in the last third of gestation, and 5% extra is allocated for adult bovines with horns. In Australia, there is a recommended average loading rate for cattle of various liveweights regardless of whether they are pregnant or horned. For a 250 kg animal the space allocation is 0.77 m^2/animal (SCARM, 2002). The space allowance for heavier cattle is shown in Table 2.5.2.

Table 2.5.2 Recommended average loading rates for Australian cattle of various liveweights (SCARM, 2002)

Mean liveweight of cattle (kg)	Floor Area (m^2/head)
250	0.77
300	0.86
350	0.98
400	1.05
450	1.13
500	1.23
550	1.34
600	1.47
650	1.63

(vi) ***Distance and duration***

The time a journey takes is generally more important than the distance covered (Warriss, 1990). Due to the distances that livestock are required to be transported in Australia, it is imperative to keep the total journey duration to a minimum. The land transport code of practice for cattle (SCARM, 2002) advocates that cattle can be transported for up to 36 hours but this can be extended to 48 hours if the cattle are healthy and not exhibiting signs of fatigue or stress, the weather conditions are favourable prior to and during transport, they are not lactating, pregnant or less than 6 months of age.

While the Australian codes of practice permit transport for up to 48 hours, it is recommended not to have the truck stationary for extended periods, as cattle become restless and agitated, thus compounding the potential stress caused by transport. Under the livestock transport code it is not necessary to provide animals with a rest break during long distance transport, apart from the regulation on maximum duration. The work of Fisher et al. (1999) in New Zealand reported that on long journeys even when rest stops are made

during which adequate feed and water is available, dairy cattle do not always fully recover during the rest period. Depending on the fitness of the cattle to travel, once the animals are on the truck, they are potentially able to be kept moving and transported to their destination without benefiting from a break, providing the duration of the journey is in line with the codes of practice.

The effect of transport durations of up to 24, 31 and 36 hours in cattle was investigated by (Knowles et al., 1999; Tadich et al., 2000; Tarrant et al., 1992). The physiological measurements and impact of journeys of this length is discussed in Section 2.4. Knowles et al. (1999) transported cattle for up to 31 hours, this included a 1 h rest stop on the truck after 14 hours. Conclusions indicated that a journey of 31 hours duration was not too physically demanding, as the large majority of animals had made a substantial recovery 24 hours after transport with *ad libitum* access to hay and water. It should not be interpreted however, that there was not a physiological cost to the animals being transported for up to 31 hours. Conversely, Tarrant et al. (1992) reported that steers that were transported for 24 hours suffered significant levels of dehydration and were noticeably tired upon arrival at their destination. Similarly, 36 hours of transport with a resting period was also detrimental to cattle welfare (Tadich et al., 2000). There was no mention made in this study whether an in-transit rest stop enhanced the capacity of the animals to recover from transport.

It can be concluded that mature healthy cattle can withstand journey durations of up to 31 hours, nevertheless, it should not be assumed that all types of cattle can endure durations up

to 31 hours. Many factors should be assessed before proceeding on a journey of such duration. For example, consideration must be given to the transport conditions discussed above, such as physical condition, age, pregnancy status, preparation prior to transport, expected environmental conditions during the journey, stocking density, driver quality and road conditions. Whatever the duration or distance stock are to be transported, good monitoring of the animals with inspections of adequate frequency are required (Broom, 2003).

2.6 Recovery

Unloading can present a particular risk to stock as generally they are tired from the physical effort required during transport to maintain footing, posture and also competing for space with other animals. Fatigue will be more noticeable in cattle that have undertaken trips of 24 hours or more (Knowles et al., 1999). As it is more difficult for livestock to walk down, rather than up slopes (Tarrant and Grandin, 2000; Kenny and Tarrant, 1987[a]), careful quiet handling at unloading of animals is imperative. To compound the effect of unloading it generally occurs in unfamiliar surrounds with unfamiliar handlers, further adding to the stressfulness of the situation. The period of time it takes for animals to recover from transport will be dependent on the condition of the animals and their preparation prior to transport and the transport conditions and duration.

The time taken by animals to recover from the effects of journeys can be determined by the time taken for the physiological variables to return to their pre-transport levels (Knowles et al., 1999). Warriss et al. (1995) and Knowles et al. (1999) reported that it took between 1-5

days for mature cattle to fully recover from transport durations of up to 31 hours. The biochemical measurements recorded in both studies had fully recovered by 48 hours into the recovery period. Knowles et al. (1999) reported that cattle that had been transported for up to 31 hours, their levels of plasma BHB and urea had returned to pre-transport levels after 24 hours. Other variables, such as those associated with hydration status (osmolality, total protein and albumin) had returned to pre-transport levels after 36-48 hours of recovery (Warriss et al., 1995; Knowles et al., 1999). Recovery to pre-transport liveweights took longer than the blood measures, taking up to 5 days to regain their pre-transport liveweights. The cattle utilised in the experiments were fed good quality hay and had access to water prior to being transported. If animals have full rumens prior to being transported, their ability to recover may be enhanced (Warriss et al., 1995).

2.7 Conclusion

Our knowledge of Australian road transport practices and their impact on the animal's well-being is relatively limited. This places the Australian livestock transport industry at risk of increased scrutiny from animal welfare interest groups because there is relatively little scientific evidence to support and validate Australia's current practices. This represents a knowledge gap. The land transport code of practice for cattle (SCARM, 2002) is clear in its recommendations for the welfare of animals that are subjected to long distance transport. However, the basis for the recommendations in the code is somewhat unclear.

This literature review has detailed useful behavioural and physiological measures that have been utilised (Table 2.4.1) to measure the effects of road transport practices on animal welfare. Measuring the behavioural and/or physiological adjustments that an animal has to make to cope with its environment provides a useful structure for underpinning the assessment of an animal's welfare during transportation (Knowles and Warriss, 2000).

The work reported in this thesis was undertaken in order to investigate the impact of specific livestock transport practices on cattle welfare. Transport loading treatments and a 6 hour road transport journey focusing on the physiological responses of yearling cattle were examined. A final study was then conducted concentrating upon transport journey durations. The aim is to examine the responses of *Bos indicus* x *Bos taurus* heifers transported for journey durations of 6, 12, 30 and 48 hours.

The objective of the two studies is to scientifically validate and more clearly define how healthy cattle respond and cope with differing loading treatments and journey durations during transit and subsequent recovery under Australian conditions.

Chapter 3

3. THE EFFECT OF LOADING PRACTICES AND 6 H ROAD TRANSPORT ON THE PHYSIOLOGICAL RESPONSES OF YEARLING CATTLE

3.1 Introduction

There is increasing public concern for the welfare of livestock during transport (Knowles and Warriss, 2000), as transportation has the potential to expose animals to significant levels of physical and emotional stress. Potential stressors during transportation include loading and handling, changes in social grouping, driving practices, transport environmental conditions and duration. While cattle may vary in their ability to cope with such stressors, their capacity to cope is still likely to decrease as stressors are combined or as the magnitude of individual stressors increases.

The response to transport is influenced by the methods used particularly during handling and loading (Broom, 2003[b]). However, there are very few reports of the effects of different loading practices on the response of cattle to transport. Most of the existing knowledge centres around the effects of transport itself, rather than upon the methods used during handling and loading.

Transport operators in Australia commonly utilise an electric goad or prodder when loading livestock. Whilst the use of such devices should be kept to a minimum (Grandin, 1997) the

additional effect of this practice on animals during loading is not well understood. Therefore, the aim of the present study was to determine the impact of this practice on the physiological responses in cattle during the early stages of transport and to quantify the physiological responses of cattle during a 6 h road transport journey with a post-transport recovery of 17 h.

3.2 Materials and Methods

Cattle

Sixteen Angus yearling steers were purchased from a local property and maintained on pasture at the FD McMaster Laboratory Chiswick, Armidale, New South Wales for 3 weeks prior to the commencement of the experiment. The liveweight of the steers was 299 ± 2.6 kg.

Vehicle

A standard 16 tonne fixed chassis stock truck was used for the experiment. The stock crate was modified such that four individual pens were built within the first two compartments of the crate (see Figure 3.1). All four pens were identical in their design (2.4m long x 0.7m wide) allowing each animal to stand parallel with the direction of travel. This facilitated a safe environment for the animals and staff during the regular blood sampling throughout the transport phase. The crate was also fitted with a temperature and humidity logger (Tiny Tag plus TGP1500 loggers, Omni Instruments, Scotland, UK). A temperature and humidity index (THI) was calculated from the temperature and humidity data downloaded from the loggers according to the formula of West (1994).

Figure 3.1 Modifications to stock crate and cattle position during transport

Experimental design

The experiment was approved by the Animal Ethics Committee of the CSIRO Livestock Industries FD McMaster Laboratory (AEC No. 04/41). Sixteen animals were randomly allocated to one of two loading treatments and there were 4 journey replicates. The cattle in one treatment group received 4 consecutive prods (1-2 s duration/prod) using an electric stock prod (The McGrath Company, Nebraska) in the handling race immediately before being quietly loaded, whilst the other group (control) received no prodding and were quietly loaded onto the truck. The animals were in single file in the handling race and did not have a chance to interact with other cattle after their prodding treatment and prior to being loaded onto the truck. The experiment was conducted and repeated over 4 consecutive days, where 4 animals/day were transported for 6 h over a total distance of 273 km. The animals were not fasted before transport. They were quietly walked into the

yards before loading. The truck journey consisted of a 25 min circuit which facilitated collection of blood samples every 30 min. The transport phase commenced at 09:00 each day.

After completion of transport (15:00), the cattle were unloaded quietly and held in a holding pen for 6 h with access to hay and water. After this period (21:00), they were moved to an adjoining empty pastured paddock with access to water. On the following morning (08:00), the cattle were returned to the yards for removal of temperature loggers and final blood sampling

Blood sampling and biochemical measurements

The cattle had an intravenous jugular catheter inserted under local anaesthetic the day before transport. An external tube (5 mm diameter) was placed over the catheter and this was taped to the dorsal aspect of the neck. Blood samples were collected via the catheter before loading (-1 h), at the commencement of loading (0 h), during transport (0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5 and 6 h), and after transport (7, 9, 12 and 23 h). Two blood samples were collected at each of these time points in serum and EDTA monovettes (10mL) (Sarstedt, Germany). They were taken into the laboratory and left at room temperature. Prior to centrifuging, whole blood was analysed for haematological variables (white blood cells (WBC), neutrophils, lymphocytes, monocytes, eosonophils, basophils, red blood cells (RBC), haemaglobin (HGB), haematocrit (HCT)) using a haematology auto-analyser (Cell-Dyn 3500R, Abbott Diagnostics, California, USA).

Both tubes were then centrifuged (3000 rpm for 10 min at 4°C) and the serum and plasma were harvested and frozen (-20°C) until they were required for analysis. Serum samples were analysed for cortisol concentration and osmolality and the plasma samples were analysed for total protein, blood urea nitrogen, creatine kinase and haptoglobin concentrations. Haptoglobin, osmolality and haematology were assessed at 5 time points (-1, 1, 3, 6 and 23 h).

Plasma cortisol concentrations were determined using a commercial radioimmunoassay (Spectria Cortisol RIA, Orion Diagnostica, Espoo, Finland).

Blood urea nitrogen (BUN), creatine kinase (CK) and total protein (TP) plasma concentrations were measured using a clinical autoanalyzer (DADE Behring Diagnostics ACA, Walton Manor, United Kingdom). Osmolality was measured using a vapour pressure osmometer (Wescor, 5500 XR, Labequip, Ontario, Canada). Haptoglobin concentration was analysed using the method of Jones and Mould (1984), modified to account for the effect of free haemoglobin due to haemolysis (Slocombe and Colditz, 2005).

Body temperature

On the day prior to transport, cattle were fitted with temperature loggers and harnesses to record body temperature. Temperature loggers (Minilog M108-TXC, Vemco Ltd., Nova Scotia, Canada) were fitted onto probes (18 cm long x 1 cm diameter) that were placed in the rectum and secured in place via elastic cord attached to a girth harness (see Figure 3.2).

The rectal probes allowed normal defecation by the animals. Body temperature was recorded every 3 min until the harnesses and probes were removed 23 h after commencement of the transport.

Figure 3.2 Cattle fitted with harnesses and rectal temperature loggers

Statistical Analysis

Blood parameters were normalized prior to the analysis where necessary. Log transformations were used for neutrophils, lymphocytes, monocytes and eosinophils counts, haemoglobin concentration, haematocrit %, creatine kinase and blood urea nitrogen concentrations, while the square root transformation was used for basophile count. The data were analysed using the mixed model procedure in *SAS* (*SAS* Institute Inc., Cary, North Carolina). The model contained the fixed effects of loading treatment (prod vs control), journey replicate (Day), blood sampling time (Time) and their interactions plus animal as the random term. For body temperature, the measurement that coincided with each of the 18 blood sampling times was extracted from the average temperature profiles and

subsequently analysed. The aim of the statistical analyses was to examine the effect of loading with an electric prodder on the response of cattle to transport, and changes in physiological variables associated with 6 h of transport.

3.3 Results

The on-board temperature conditions varied between the days of the study (Figure 3.3). The cooler temperatures experienced on day 4 coincided with rain and overcast conditions. The THI ranged from 44-80 over the 4 day period.

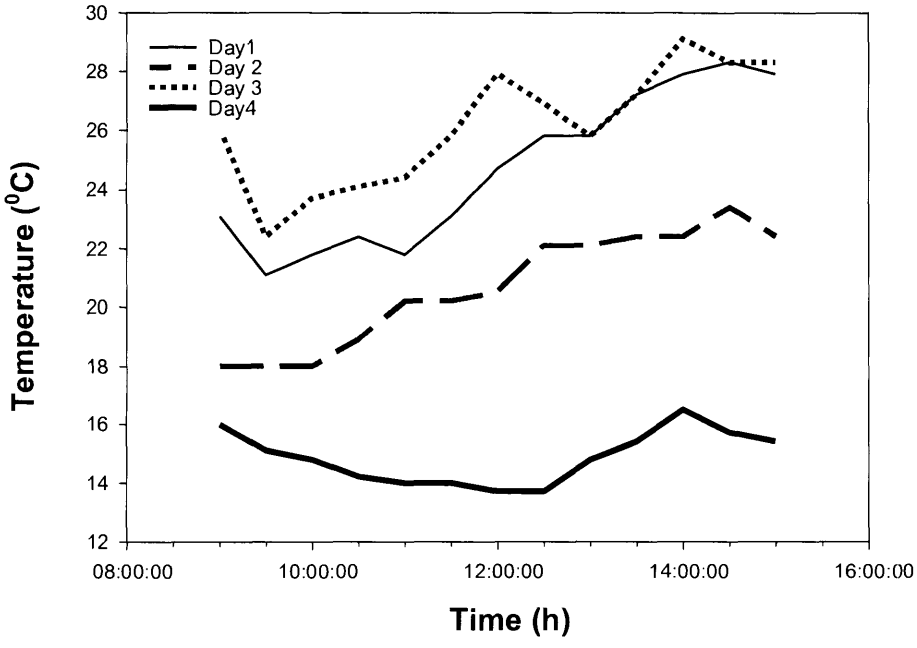


Figure 3.3 Ambient temperature profiles (in crate) during the 6 h of transport over the four days

(i) ***Effect of loading treatment***

Loading treatment did not significantly affect rectal temperature, nor did it affect any of the variables measured (Tables 3.1 and 3.2, Figure 3.4). For WBC (Table 3.2), the effect of the loading treatment interacted with the day of transport ($P<0.05$). On days 1 and 2, WBC were greater in the groups receiving electric prodding during loading, while on days 3 and 4, WBC were greatest in controls. In addition, a significant ($P<0.01$) treatment × day × time interaction was observed for plasma total protein (Table 3.1).

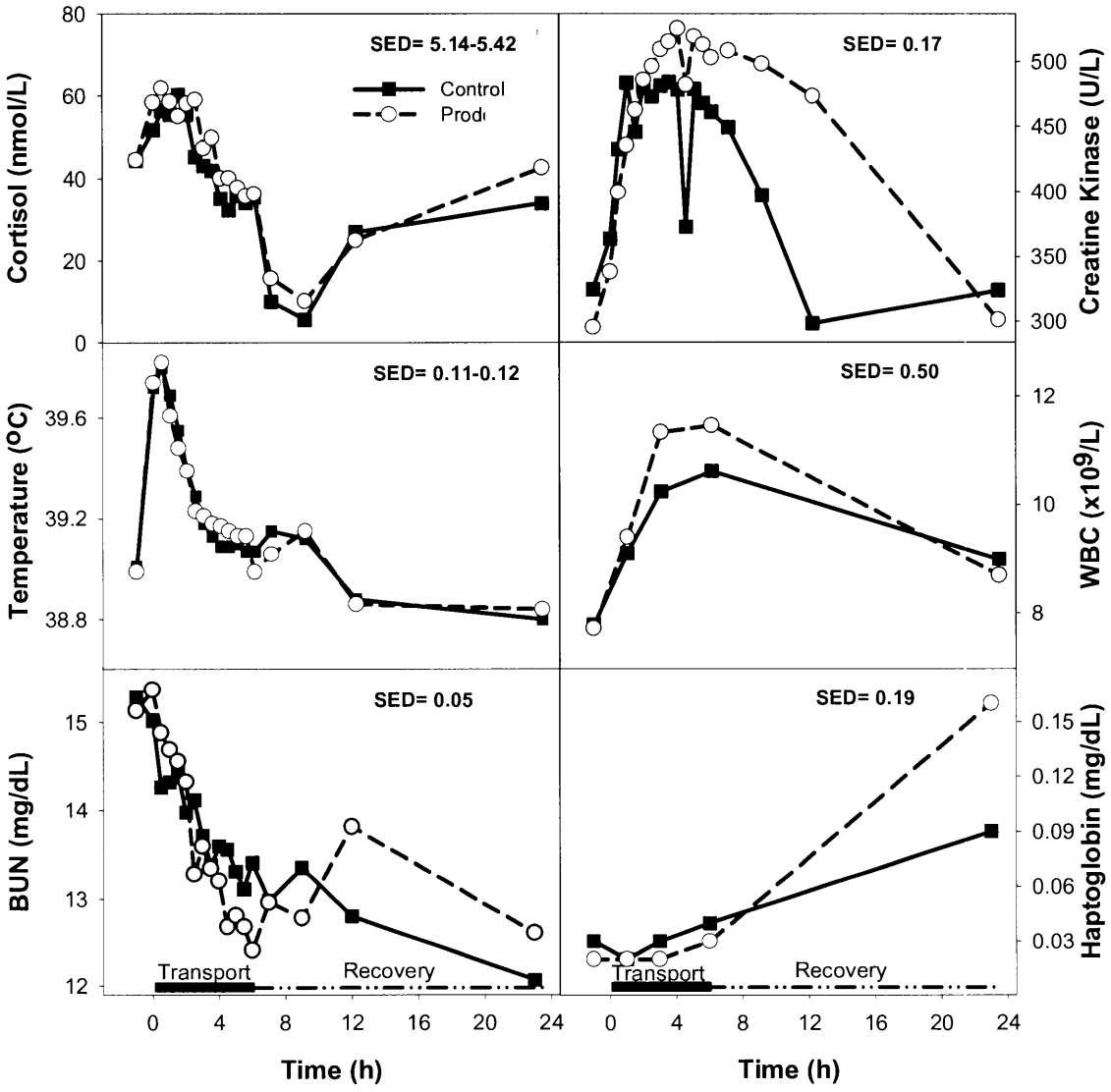


Figure 3.4 Differences between loading treatment groups for cortisol, creatine kinase, temperature, white blood cell count (WBC), blood urea nitrogen (BUN) and the acute phase protein haptoglobin in response to 6 h transport and during 17 h of recovery

Table 3.1 Effect of loading treatment, day, sampling time and their interactions on body temperature (Temp.) and plasma concentrations of cortisol (nmol/L), total protein (g/dL), creatine kinase (CK U/L) and blood urea nitrogen (BUN mg/dL) of yearling steers transported for 6 h.

	Cortisol	Total Protein	CK†	BUN†	Temp.
Main Effects					
Load. Treat. (LT)					
Control	39.11	6.79	426.75	13.67	39.23
Prod	43.14	6.94	453.87	13.58	39.23
SED	5.60	0.1	0.16	0.06	0.12
Significance	*ns*	*ns*	*ns*	*ns*	*ns*
Day					
1	36.07	6.93	439.88	15.13	39.41
2	43.03	6.92	529.38	13.23	39.15
3	45.22	6.88	409.12	13.49	39.22
4	40.19	6.72	393.78	12.76	39.14
SED	7.90-7.93	0.14	0.22	0.09	0.18
Significance	*ns*	*ns*	*ns*	*ns*	*ns*
Time (h)					
-1	44.49	6.78	309.76	15.21	38.99
0	55.05	6.82	350.69	15.19	39.73
0.5	59.10	6.72	415.84	14.57	39.81
1	56.98	6.72	459.25	14.51	39.65
1.5	57.28	6.70	454.46	14.44	39.52
2	56.74	6.77	483.67	14.14	39.39
2.5	52.57	6.73	486.00	13.64	39.26
3	45.31	6.77	498.95	13.65	39.20
3.5	46.13	6.84	503.56	13.35	39.16
4	37.68	6.86	507.20	13.35	39.13
4.5	36.26	7.02	438.39	13.11	39.12
5	36.73	6.93	504.26	13.04	39.12
5.5	34.83	7.00	496.36	12.92	39.10
6	35.92	7.13	487.26	12.90	39.03
7	12.99	7.08	483.23	12.97	39.10
9	7.96	6.95	447.73	13.06	39.13
12	25.97	6.93	375.93	13.29	38.86
23	38.30	6.77	312.37	12.34	38.82
SED	3.76-3.85	0.09	0.11	0.03	0.06
Significance	***	***	***	***	***
Interactions					
LT × Day	*ns*	*ns*	*ns*	*ns*	*ns*
Day × Time	*	**	*ns*	*ns*	**
LT × Time	*ns*	*ns*	*ns*	*ns*	*ns*
LT × Day × Time	*ns*	**	*ns*	*ns*	*ns*

†Back transformed means shown; ns – not significant; SED – Standard error of the difference
***P<0.001, ** P<0.01, * P<0.05

Table 3.2 Effect of loading treatment, day, sampling time and their interactions on haematology, osmolality and haptoglobin concentration of yearling steers transported for 6 h

	WBC	NEU†	LYM†	MON†	EOS	BAS†	RBC	HGB†	HCT†	Osmol.	Hapt.†
Main Effects											
Load. Treat. (LT)											
Control	9.34	4.21	3.57	0.77	0.11	0.09	7.32	11.13	29.49	279.52	0.03
Prod	9.72	4.18	3.85	0.77	0.07	0.11	7.70	11.52	30.76	278.18	0.04
SED	0.52	0.12	0.13	0.14	0.04	0.02	0.33	0.02	0.02	1.49	0.21
Significance	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
Day											
1	8.50	4.27	3.29	0.70	0.04	0.07	7.26	11.29	30.37	282.85	0.03
2	9.27	3.65	3.90	0.80	0.08	0.11	7.87	11.55	30.63	269.85	0.04
3	9.15	3.58	3.82	0.77	0.08	0.10	7.21	10.95	29.32	269.68	0.03
4	11.21	5.57	3.88	0.82	0.17	0.13	7.71	11.50	30.16	292.85	0.04
SED	0.74	0.17	0.18	0.20	0.06	0.03	0.47	0.03	0.03	2.11	0.30
Significance	*	*ns*	*ns*	*ns*	*ns*	*	*ns*	*ns*	*ns*	*	*ns*
Time (h)											
-1	7.75	2.24	4.23	0.65	0.10	0.08	7.94	11.98	31.93	276.12	0.03
1	9.25	4.27	3.75	0.68	0.08	0.13	7.54	11.38	30.17	279.56	0.02
3	10.79	6.27	3.29	0.76	0.05	0.13	7.22	10.88	28.76	279.69	0.03
6	11.04	6.48	3.25	0.88	0.008	0.10	7.33	11.03	29.24	280.50	0.04
23	8.84	3.36	4.15	0.92	0.22	0.07	7.53	11.37	30.58	278.37	0.12
SED	0.39	0.11	0.07	0.09	0.03	0.01	0.11	0.01	0.01	1.17	0.16
Significance	***	***	***	***	**1*	***	***	***	***	*ns*	***
Interaction											
LT × Day	*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
Day × Time	*	*ns*	*ns*	*ns*	*ns*	*ns*	*	**	*	***	*ns*
LT × Time	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
LT × Day × Time	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*

†Back transformed means shown, *** P<0.001, ** P<0.01, * P<0.05; ns – not significant; SED – Standard error of the difference

WBC- white blood cell count (x 10^9/L), NEU- neutrophils (x 10^9/L), LYM- lymphocytes (x 10^9/L), MON-monocytes (x 10^9/L), EOS-eosinophils (x 10^9/L), BAS-basophils (x 10^9/L), RBC-red blood cell count (x 10^{12}/L), HGB-haemoglobin (g/dL), HCT- haematocrit %, Osmol.-osmolality (mOsmol/L) and Hapt.-haptoglobin (mg/dL)

(ii) ***Effect of 6 h transport***

The transport-mediated changes in total protein, osmolality, haemoglobin and haematocrit % were generally small, indicating that 6 h of transport had a minimal effect on the hydration status of the animals. The change in these variables after transport ranged from 1-9% (Table 3.3). Plasma blood urea nitrogen (BUN) significantly declined during the 6 h of transport (Figure 3.4). The stress indicators of plasma cortisol and body temperature showed similar response patterns particularly during the loading and initial stages of transport (Figure 3.4). Loading elicited sharp rises in both temperature and cortisol followed by a gradual attenuation of the response during the course of the 6 h of transport. At the conclusion of the transport phase, the levels for both cortisol and body temperature had returned to, at or below their pre-transport levels. The plasma concentration of creatine kinase rose steadily over the 6 h of transport indicating some muscle activity and damage (Figure 3.4).

Table 3.3 Percentage change in selected physiological measurements in yearling steers after 6 h transport*

Measurement	Change
WBC (x 10^9/L)	42.5 % increase
RBC (x 10^{12}/L)	7.7 % decrease
Haemoglobin (g/dL)	7.9 % decrease
Haematocrit %	8.4 % decrease
Osmolality (mOsmol/L)	1.6 % increase
Haptoglobin (mg/mL)	33.3 % increase
Cortisol (nmol/L)	19.3 % decrease
Total protein (g/dL)	5.2 % increase
Creatine kinase (U/L)	57.3 % increase
BUN (mg/dL)	15.2 % decrease
Temperature (^{o}C).	minimal change

*Initial and final values were taken from the -1 h and 6 h least square means, respectively.

WBC – white blood cell count (x 10^9/L), RBC – red blood cell count (x 10^{12}/L), BUN – blood urea nitrogen (mg/dL)

(iii) ***Post-transport recovery***

Twenty three hours after the commencement of transport, most measurements had returned to their pre-transport levels. There were exceptions, such as the acute phase

protein haptoglobin where the 23 h concentration was some 3.7 times higher than that observed prior to transport.

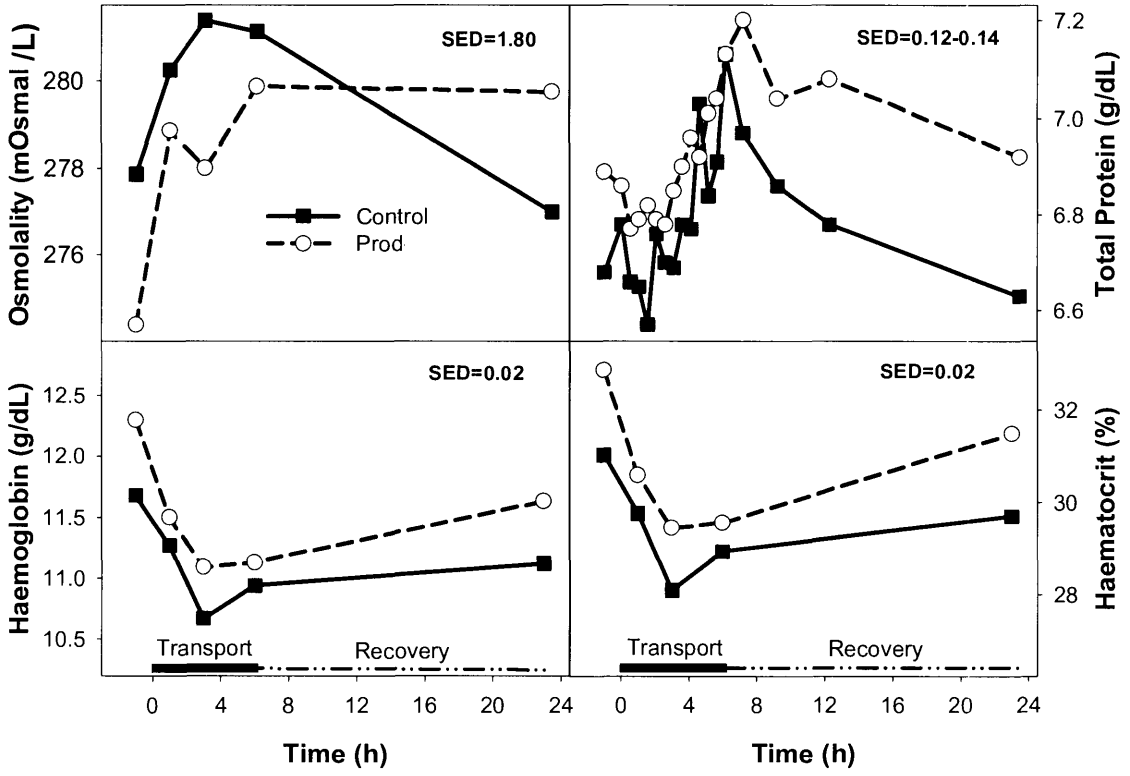


Figure 3.5 Differences between the loading treatment groups for total protein, haematocrit (HCT), haemoglobin (HGB) and osmolality

3.4 Discussion

The main finding of this study was that loading with an electric prodder did not significantly modify the physiological responses of cattle to the loading event or 6 h of subsequent road transport. Secondly, 6 h of transport induced only mild and transient

changes in physiological variables indicative of stress. A physiological response was exhibited at loading after which the cattle rapidly habituated to the transport conditions.

The absence of an effect due to use of an electric prodder does not imply that the use of such devices is desirable. Use of the electric prodder may transiently alter animal behaviour, possibly making some animals more agitated and difficult to handle. The specific effects of the use of electric prodders on the behavioural and physiological responses in cattle were not examined in the current study. It is possible that the specific effect of the electric prodder *per se* may have been overridden by the significant physiological response that occurred during loading and handling. There was no evidence that the level of stress engendered by the electric prod was detrimental to animal welfare as measured by the physiological responses in this experiment.

There was an increase in blood cortisol and body temperature during loading and the initial 2-3 h of transport, indicating that these stages were stressful to the cattle. Cortisol concentrations similarly increased in cattle transport studies by Warriss et al. (1995); Grandin (1997) and Knowles et al. (1999) in response to loading and also during the initial stages of the journey. However, the decrease in the cortisol and temperature responses during the journey indicates that the cattle rapidly habituated and adapted to the transport conditions. This is in agreement with the findings of similar studies conducted by Warriss et al. (1995) and Lay et al. (1996). The return to low values during the recovery phase may reflect the underlying circadian effect on serum cortisol. It was observed during the experiment that the regular sampling did not appear to alter the general adaptation response

of the 16 animals. Grandin (1997) reported that cattle become accustomed to repeated non-aversive procedures such as blood sampling.

In this study, unloading did not elicit any major change in either cortisol or temperature. These observations are consistent with those from other transport studies in cattle (Eldridge et al., 1988; Warriss et al., 1995) and sheep (Broom et al., 1996). Together, the results suggest that loading and the initial transport stages are the most stressful periods during transport of several hours duration and that cattle can adapt to transport of short to moderate durations.

The cortisol and sympathoadrenal responses associated with loading and the 6 h of transport are likely to account for the increase in total white blood cell and neutrophil count. Transport and handling stress have been observed to alter numerous blood cell components (Schaefer et al., 1997). Kent and Ewbank (1983) reported increases in the number of total white blood cells and neutrophils in young calves up to 6 months of age transported by road for 6 h. The increase in neutrophils and decrease in lymphocytes and eosinophils during transport are typical of a physiological response (Cole et al., 1988). Whilst there was a small increase and/or decrease in these parameters in this experiment they did not pose a significant immunological concern for the animals. Colditz et al. (2006) reported similar findings although the differential counts were much higher when cattle were trucked over a much greater distance and handled significantly more than in the present study. Almost all the blood values measured in this study had returned to pre-transport levels after 17 h of recovery.

The rise in CK over the 6 h of transport can be attributed to the muscular exertions during loading and the maintenance of balance during transport. Creatine kinase is an enzyme associated with energy metabolism in muscle which is released following a change in the permeability or damage to muscle cell membranes (Knowles and Warriss, 2000). Warriss et al. (1995) showed that plasma concentrations of CK were positively associated with journey length (5-15 h) and reported a 270% increase in CK levels over 5 h of transport. This is markedly greater than that observed in the present study (30%). Also important to note is the fact that at the completion of every 25 min circuit the truck was stationary for periods of 5 – 10 min whilst blood samples were collected, therefore the animals did not have to utilise energy or muscles maintaining their balance. These may have been factors that contributed to the lower CK concentrations recorded in this experiment. The CK concentration had returned to pre-transport levels after 17 h of recovery, indicating that there was not a sustained effect on muscle function.

The small changes in serum osmolality, total protein, haemoglobin and haematocrit % (Figure 2) indicate that 6 h of transport had minimal impact on the hydration status of the cattle. Total protein concentrations, osmolality, haemoglobin and haematocrit had almost fully recovered by 17 h post transport. The return of blood values to control levels within a relatively short time during recovery suggests that dehydration was minimal. Any slight dehydration that did occur during the 6 h journey duration was resolved by access to water during the recovery phase. In addition, there was no evidence of any transport and/or fasting-induced protein catabolism as reflected by increases in blood urea nitrogen. Indeed, the significant decrease in blood urea nitrogen during transport in the present study, where

cattle were not fasted before transport, contrasts with the 6.5% increase observed by Warriss et al. (1995) after 5 h transport in cattle. It was not stated whether cattle were fasted before transport in the study by Warriss et al. (1995).

There was a significant increase in serum haptoglobin levels from the beginning of transport (2.09 mg/dL) to the end of the recovery phase (12.07 mg/dL). Haptoglobin is an acute phase protein that appears in the blood in response to tissue injury and inflammation (Murata and Miyamoto, 1993). It was reported by Arthington et al. (2003) that cattle have elevated blood concentrations of acute phase proteins in response to stressful stimuli. Because haptoglobin levels in serum can remain elevated for over 4 days following a short period of activation of the acute phase response, it can be a valuable marker of physiological responses (Peppard et al., 1994). In this experiment, the 23 h measurement of haptoglobin was significantly greater than pre-transport values for the control and treated groups, indicating that trucking activated acute phase proteins and providing further evidence that a mild physiological response did occur.

3.5 Conclusion

This is the first study to show the moderate use of an electric prodder during loading does not modify the physiological responses of cattle to loading or subsequent road transport for 6 h. Furthermore, loading and the initial 2-3 h of transport induced a moderate physiological response, however, cattle habituated to the journey conditions and most physiological indicators of physiological responses returned to baseline during the 17 h

recovery. The results suggest that a road journey of 6 h during temperate environmental conditions does not provide a significant stress challenge to healthy yearling cattle.

Chapter 4

4. THE EFFECT OF ROAD TRANSPORT DURATION ON INDICATORS OF CATTLE WELFARE

4.1 Introduction

Nearly all Australian cattle are transported at least once during their lives. This occurs when they are moved from one farm to another, to saleyards, feedlots and/or abattoirs (Matthews et al., 2000). Transport represents a critical phase in animal production as it has the potential to negatively impact on product quality thus resulting in economic loss, and animal welfare. Therefore, monitoring, maintaining and improving standards during livestock transport is imperative to optimise animal welfare and economic outcomes.

In order to improve the welfare of livestock during transport, particularly over long durations, it is necessary to make measurements which allow the assessment of welfare (Broom, 2000). These might include environmental conditions, journey characteristics and psychological, behavioural and physiological responses (Knowles, 1999; Grandin, 1997).

Whilst studies in the United States (US) and the European Union (EU) have focused on the effects of transport duration on calves and mature cattle (Cole et. al., 1988; Kent and Ewbank, 1983; Tadich et al., 2000; Warriss et. al., 1995; Knowles et. al., 1999), very little is known of the impact of transport duration in Australian conditions. In previous transport duration studies conducted overseas, the maximum journey duration was 36 h (Tadich et

al., 2000). The results from this study indicated that journeys of this length may be detrimental to cattle (Tadich et al., 2000). Knowles et al. (1999) concluded that cattle could be transported for durations of up to 31 hours, however, 24 hours may be more appropriate given their observations that nearly half of the animals began to lie down after this time. In Australia, under the model code of practice for land transport of cattle (SCARM, 2002), mature non-lactating cattle can be transported for a maximum of 36 hours, with an extension to 48 hours if the animals are travelling well and not showing signs of fatigue, thirst or distress. The extension up to 48 hours is permissible if there is no prediction of adverse weather conditions and the entire journey can be completed within 48 hours. The animals are then required to be rested with water and feed for at least 18 hours immediately upon arrival. It is understood that this specification was based on the maximum allowable time for water deprivation in cattle. Importantly, there is no scientific evidence to substantiate whether the maximum transport duration allowable under the code is valid.

In view of this knowledge gap, this study was undertaken to quantify the responses of *Bos indicus* x *Bos taurus* heifers transported for journey durations of 6, 12, 30 and 48 h.

4.2 Materials and Methods

This experiment was approved by the Animal Ethics Committee of the CSIRO Livestock Industries JM Rendel Laboratory (AEC No. RH210/05). The experiment was conducted during September and October 2005.

Cattle

The cattle used for the experiment were sourced from a Queensland pastoral company. Four hundred and eighty *Bos indicus* x *Bos taurus* heifers were bred on two company properties. The two properties were located in central western Queensland (QLD) 120 km south and 66 km south west of Boulia, respectively.

Eight weeks before the commencement of the experiment both groups (Group A and B) of 240 cattle were transported from their property of origin to Winton which took approximately 6-7 h.

Upon arrival at Winton, each group was unloaded and provided a rest period of 15-20 h and then reloaded onto the same trucks to complete the balance of the journey through to the backgrounding property near Roma in south western QLD. This was an additional distance of approximately 900 km. The journey took 10-15 h depending upon the compulsory rest periods taken by the individual drivers.

The two groups were initially kept separate and then mixed together in August 2005. The combined mob (Groups A and B) were then randomly allocated to the two transport replicate groups of 240 animals (Replicate 1 and 2) and maintained on mature Buffell grass (*Cenchrus ciliaris*) pastures. The pastures also contained lesser amounts of Flinders (*Iseilema spp.*) and Mitchell (*Astrebla spp.*) grass. The replicate groups were left to graze these paddocks for a period of 4 weeks to allow recovery and socialisation prior to the

commencement of the transport study. The cattle ranged in weight from 350 to 400 kg and were 18 to 24 months of age.

Transport vehicle

The livestock transport vehicles used in this experiment were typical for the Australian livestock transport industry (Figure 4.1). A two-deck Byrne cattle trailer which had two pens (6.1m x 2.5m) per deck was utilised.

Figure 4.1 Commercial livestock carrier used in the study

Cattle were transported at a stocking density of approximately $1m^2$ per animal. The land transport code of practice for cattle (SCARM, 2002) recommends a stocking density 0.98-$1.05m^2$ per animal for cattle that weigh between 350 and 400 kg.

A temperature and humidity logger (Hygrochron Temperature/Humidity Logger iButton DS1923, Maxim Integrated Products, Dallas, Texas) was fitted in the centre rail on the

bottom deck (Figure 4.2). The logger unit was programmed to record temperature and humidity every 4 minutes. A temperature and humidity index (THI) was calculated from the temperature and humidity data downloaded from the loggers according to the formula used by West (1994).

Figure 4.2 Placement of the temperature and humidity logger inside the stock crate on the lower deck

A global positioning system (GPS) unit (GPS 60, Garmin International Inc., Kansas) was placed in the cabin of each truck so that journey duration, movement and the route travelled could be tracked for each transport duration treatment. The GPS unit was programmed to capture data every 2 min.

Experimental design

The experimental design which encapsulates the flow of cattle from the backgrounding property to the feedlot is illustrated in Figure 4.3.

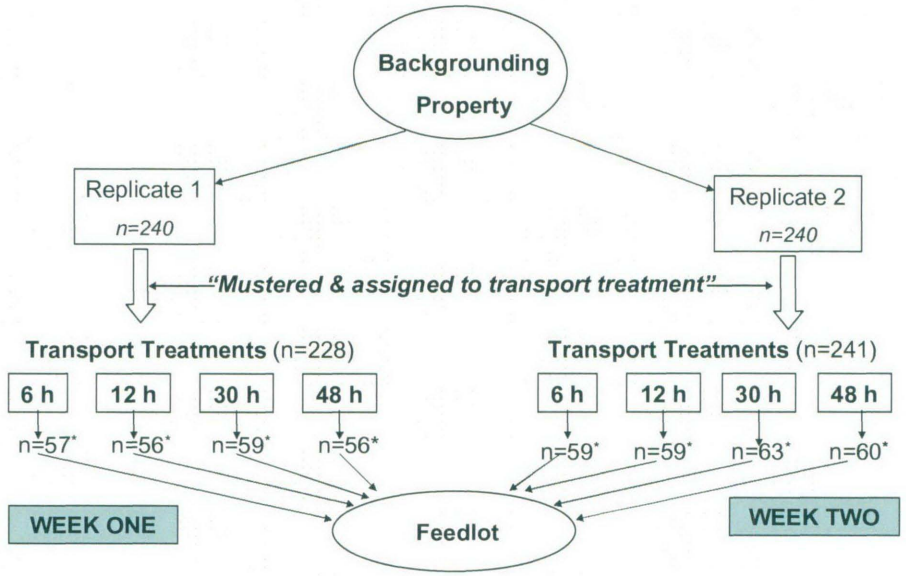


Figure 4.3 Experimental design from the backgrounding property to the feedlot

Four transport duration treatments of 6, 12, 30 and 48 h were utilised for each replicate and the experiment was conducted over two consecutive weeks. On each week, the day before the first journey, each replicate group was brought into the yards. The animals were weighed and randomly allocated to their duration treatments after stratification for liveweight. A total of 56-63 animals were allocated to each transport journey and of these, 15 focal animals/journey were randomly selected. Detailed measurements were taken on the focal animals.

Once the animals had been assigned to their transport duration treatments, they were returned to holding paddocks which adjoined the cattle yards. The holding paddocks contained similar pastures to those described previously. Having the cattle nearby in these holding paddocks facilitated ease of movement into the yards on each day of transport. The departure of four transport treatments was staggered so that all vehicles arrived at the feedlot on the same day (Table 4.1).

Table 4.1 Experimental schedule for each replicate

Task	Day and time							
	1	2	3	4	5	6	7	8
Weighed and draft into groups	**08:00**							
Departure								
48 h		**09:00**						
30 h			**10:00**					
12 h			**19:00**					
6 h				**09:00**				
Arrival								
48 h					**09:00**			
30 h					**16:00**			
12 h					**07:00**			
6 h					**15:00**			
Post-transport sampling and weighing								
48 h						**09:00**		**09:00**
30 h						**16:00**		**16:00**
12 h						**07:00**		**07:00**
6 h						**15:00**		**15:00**

The animals were not fasted prior to being loaded onto the trucks. The transport treatment groups were mustered quietly from the holding paddock approximately 2 h prior to their scheduled departure. During this time in the yards they had access to water. The focal animals were drafted off and weighed and blood sampled (via caudal or jugular venipuncture) prior to being loaded onto the truck. The focal animals were distributed evenly throughout the pens on the truck.

Upon arrival at the feedlot, all cattle were unloaded and quietly walked to the processing area. The entire group was weighed and the 15 focal animals were blood sampled. The focal animals from each transport treatment were then drafted off and held in a small holding pen adjacent to the processing area with access to feed and water for 72 h. They were fed petrel wheaten (*Triticum petrel*) hay, which is a wheat variety developed specifically for stock fodder.

Body temperature, blood sampling and liveweights

In the 2 h period prior to departure, the focal animals were fitted with temperature loggers to record body temperature. The temperature loggers (DS1921H, High Resolution Thermochron iButton Range: +15°C to +46°C, Maxim Integrated Products, Dallas, Texas) were attached to a probe (18 cm long x 1 cm diameter) that was inserted into the rectum of the animal and held in place by an elastic cord that connected to a girth strap (Figure 4.4). The rectal probes did not inhibit normal defecation. Body temperature was recorded every 3 min until the probe was removed 72 h post-transport.

Figure 4.4 Temperature loggers fitted to focal animals prior to transport

Blood samples were collected via caudal or jugular venipuncture from each of the focal animals at four time points: pre-transport (PT) and 0 (unloading), 24 and 72 h post-transport. Animal liveweights were recorded at the same times. Two blood samples (6 mL EDTA and 10 mL serum vacutainer (Becton Dickinson, New Jersey)) were collected on each occasion and kept on ice.

The 6 mL tube was packed in ice and sent to a Veterinary Pathology Laboratory in Brisbane QLD for analysis of haematology (cell counts of white blood cells (WBC), red blood cells (RBC), neutrophils (NEU), lymphocytes (LYM), monocytes (MON) eosinophils (EOS), basophils (BAS) plus haemoglobin concentration (HGB), haematocrit (HCT), mean corpuscular volume (MCV), packed cell volume (PCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC)).

The 10 mL tubes of blood were centrifuged (3200 rpm for 15 min at 4°C) and the serum was harvested and frozen (-20°C) until it was required for analysis. These samples were subsequently analysed for osmolality (OSMOL) and the concentrations of cortisol, haptoglobin (HAPT), total protein (TP), albumin (ALB), creatine kinase (CK), β-hydroxybutyrate (BHB) and blood urea nitrogen (BUN).

Serum cortisol was determined using a commercial radioimmunoassay (Spectria Cortisol RIA, Orion Dianositca, Espoo, Finland). The concentrations of BUN, CK, BHB, ALB and TP in serum were measured using a clinical autoanalyzer (DADE Behring Diagnostics ACA, Walton Manor, United Kingdom). Osmolality (OSMOL) was measured using a vapour pressure osmometer (Wescor, 5500 XR, Labequip, Ontario, Canada). Haptoglobin (HAPT) concentration was analysed using the method of Jones and Mould (1984), modified to account for the effect of free haemoglobin due to haemolysis (Slocombe and Colditz, 2005).

Behavioural measures and observations

The animals in the 12 h and 48 h transport treatment groups were monitored for specific behaviours during the first 6 h after arrival via video recording. The video filming did not commence until both groups of 15 focal animals had been placed in their recovery pens. The tapes were subsequently evaluated and specific behaviours including whether the animals were eating, walking, lying down and standing were recorded at 5 min time intervals for each group. Drinking events were also documented during 10 min intervals.

Feedlot finishing

Focal and non-focal animals were reunited into their original replicate groups at the completion of the 72 h recovery phase. The following day, the replicate group underwent the standard induction process into the feedlot. This comprised routine health treatments, assessment of dentition and recording liveweights. Each replicate was then placed in a single feedlot pen and fed for a period of 42 days prior to slaughter. The ration composition is shown in Table 4.2 and comprised 14.1% crude protein and the metaboliseable energy of 12.9 MJ/kg. During the feedlot finishing phase, the health of these animals was closely monitored. There were no reports of morbidity or other health issues for cattle in either replicate during the finishing phase.

Table 4.2 Feedlot ration for the 42 day grain-assist program

	Starter	**Intermediate 1**	**Intermediate 2**	**Finisher**
	(3 to 6 days)	(following 3 days)	(following 3 days)	(final 30-33 days)
Commodity	**%**	**%**	**%**	**%**
Sorghum	19.50%	31.00%	44.00%	55.50%
Wheat	19.50%	19.50%	19.50%	19.50%
Molasses	12.00%	11.00%	6.00%	1.50%
Liquid Supplement	3.00%	3.50%	4.50%	5.00%
Protein Meal	5.00%	3.00%	9.00%	7.00%
Silage	11.00%	11.00%	8.50%	9.50%
Cotton Seed	7.00%	7.50%	5.50%	1.00%
Cotton Hulls	12.00%	8.00%	3.00%	1.00%
Hay	11.00%	5.50%		

Statistical analysis

The data were analysed using *SAS* (*SAS* Institute Inc., Cary, North Carolina). The normality of the data was tested prior to analysis. Several blood variables were normalised via log (albumin, lymphocytes, monocytes, eosinophils, basophils, white blood cells, red

blood cells, haptoglobin, neutrophils and neutrophil:lymphocyte ratio) or square root (cortisol, β-hydroxybutyrate) transformations. Creatine kinase data was normalised using inverse log transformation.

A general linear model (ANOVA) was used to analyse the pre-transport response measures. The model contained the fixed effects of transport duration and replicate and their interaction.

For the post-transport measures, a repeated measures analysis using the mixed model procedure in *SAS* was performed. The models contained the fixed effects of transport duration, replicate, sampling time (the time blood samples were taken post-transport) and the first and second order interactions and a random term for animal.

Significant treatment differences were found for some measures prior to transport. Where this occurred, the analyses for the post-transport response measures were repeated with the pre-transport values included as a covariate in the model. The significance of the main effects and interactions did not change after fitting the covariate to the model, so the results that are presented are from the initial model, without the covariate.

A general linear model (ANOVA) was used to analyse feedlot average daily gain. The model contained the fixed effects of transport duration and replicate and their interaction.

A chi squared test was performed to analyse the behaviour data for bouts of lying, drinking and eating for each week. The analysis tested the proportion of animals in each treatment group that were performing the behaviours within the two 3-hour periods after arrival.

4.3 Results

Transport journey duration, distance travelled, moving time and the time the truck was stationary are summarised in Table 4.3 for each of the transport treatments from each replicate.

Table 4.3 Summary of transport duration for replicates 1 and 2

	Replicate1			
	Total Time	**Distance Travelled (km)**	**Moving Time**	**Time Stopped**
48 h*	**NA**	3160 km	**NA**	**NA**
30 h	29 h 52 min	2164 km	25 h 30 min	4 h 22 min
12 h	12 h 20 min	827 km	10 h 36 min	1 h 44 min
6 h	6 h 10 min	476 km	5 h 46 min	24 min
	Replicate 2			
	Total Time	**Distance Travelled (km)**	**Moving Time**	**Time Stopped**
48 h	47 h 34 min	3158 km	36 h 36 min	10 h 58 min
30 h*	**NA**	2200 km	**NA**	**NA**
12 h	11 h 42 min	760 km	9 h 48 min	1 h 52 min
6 h	6 h 12 min	444 km	5 h 54 min	18 min

NA – Not available as the GPS units were inadvertently turned off before the journey was completed.

4.3.1 Pre-transport blood chemistry and haematology

The statistical significance of the main effects and their interaction are shown in Tables 4.4 and 4.5 for the blood chemistry (BUN, CK, TP, ALB, BHB, cortisol, OSMOL, HAPT), and haematology (HGB, RBC, HCT, MCV, MCH, MCHC, PCV, WBC, NEU, LYM, MON, EOS, BAS and N:L ratio) measures, respectively.

There were significant transport duration × replicate interactions (Tables 4.4 and 4.5) for ALB, OSMOL, HCT, MCHC, NEU, LYM and N:L ratio. There were significant transport duration differences for BUN, CK, TP, BHB, PCV, NEU and BAS. Similarly there were differences between each replicate for cortisol, MCV and MON.

Table 4.4 Pre-transport differences between the transport duration groups and replicates in blood chemistry and liveweight.

	BUN	CK†	TP	ALB†	BHB†	Cortisol†	OSMOL.	HAPT.†	Liveweight
Main Effects									
Transport duration (h)									
6	13.47	163.43	70.20	37.77	0.21	117.20	293.43	0.950	370.50
12	14.30	162.36	72.93	37.96	0.20	104.77	301.20	0.960	373.100
30	13.43	194.77	71.01	37.36	0.27	116.90	294.37	0.970	368.900
48	15.40	275.00	73.43	38.65	0.27	141.40	296.43	0.950	376.230
SED	0.65	0.006	1.2	0.018	0.031	0.72	2.60	0.019	5.130
Significance	**	**	*	ns	*	ns	*	ns	ns
Replicate									
1	14.38	198.2	71.65	38.57	0.24	141.33	297.8	0.96	371.77
2	13.92	187.1	72.14	37.31	0.24	99.87	294.9	0.96	372.58
SED	0.46	0.004	0.84	0.012	0.022	0.51	1.84	0.009	3.63
Significance	ns	ns	ns	**	ns	***	ns	ns	ns
Interaction									
Transport duration x Replicate	ns	ns	ns	**	ns	ns	*	ns	ns
SED				0.003			3.67		

†Back transformed means shown, *** P<0.001, ** P<0.01, * P<0.05; ns – not significant; SED – Standard error of the difference BUN (mg/dL), CK (U/L), TP (g/dL), ALB (g/L), BHB (mmol/L), cortisol (nmol/L), Osmol. (mOsmol/L), Hapt. (mg/dL) and liveweight (kg)

Table 4.5 Pre-transport differences between transport duration groups and replicates in haematology measures.

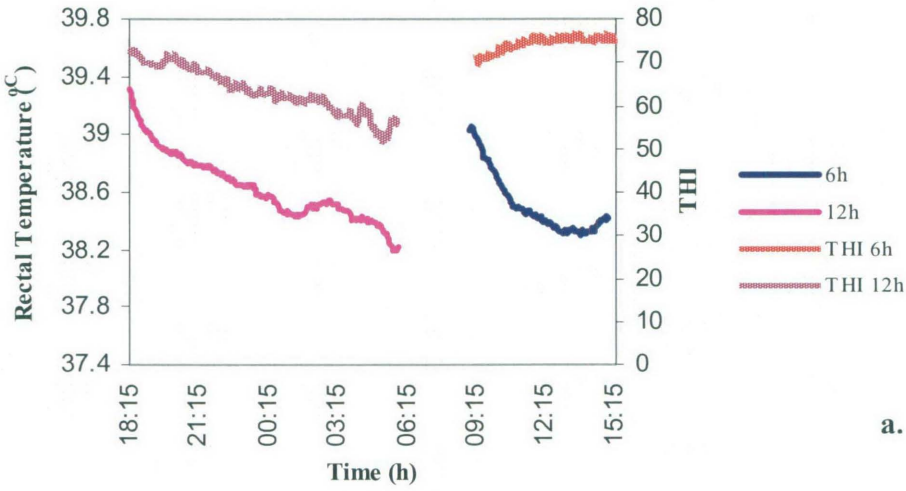
	HGB	RBC†	HCT	MCV	MCH	MCHC	PCV	WBC†	NEU†	LYM†	MON†	EOS†	BAS†	N:L† Ratio
Main Effects														
Transport duration (h)														
6	147.71	9.32	42.0	45.35	15.86	350.36	380.00	7.64	3.07	6.46	1.41	1.23	0.20	2.31
12	144.13	9.28	42.0	45.20	15.66	344.97	245.30	7.49	3.03	6.14	1.39	0.98	0.18	2.44
30	143.67	9.00	42.0	46.00	16.00	347.00	336.17	7.44	3.22	5.51	1.27	1.21	0.17	2.64
48	149.43	9.23	43.0	46.23	16.23	350.23	285.50	8.11	3.96	5.55	1.39	1.16	0.24	2.95
SED	2.71	0.03	0.008	1.02	0.36	1.8-1.18	48.70	0.058	0.066	0.10	0.088	0.16	0.10	0.067
Significance	ns	ns	ns	ns	ns	P<0.01	P<0.05	ns	P<0.001	ns	ns	ns	P<0.05	P<0.05
Replicate														
1	144.78	9.12	0.41	44.52	15.92	356.55	310.2	18.43	3.3	6.19	1.46	1.07	0.02	2.49
2	147.69	9.3	0.44	46.86	15.95	339.73	313.3	17.69	3.32	5.62	1.27	1.21	0.19	2.66
SED	1.92	0.02	0.006	0.73	0.25	1.28	34.29	0.042	0.05	0.071	0.062	0.11	0.07	0.047
Significance	ns	ns	P<0.001	P<0.01	ns	P<0.001	ns	ns	ns	ns	P<0.05	ns	ns	ns
Interaction														
Transport duration x Replicate	ns	ns	P<0.01	ns	ns	P<0.001	ns	ns	P<0.001	P<0.001	ns	ns	ns	P<0.001
SED			0.11			2.55			0.09	0.14				0.096

†Back transformed means shown, *** P<0.001, ** P<0.01, * P<0.05; ns – not significant; SED – Standard error of the difference
HGB (g/dL), RBC (x 10^{12}/L), HCT %, MCV (x $1e^{-15}$/L), MCH (x 10^{-12}/L), MCHC (g/L), PCV (μ/L), WBC (x 10^{9}/L), NEU (x 10^{9}/L), LYM (x 10^{9}/L), MON (x 10^{9}/L), EOS (x 10^{9}/L), BAS (x 10^{9}/L) and N:L Ratio

4.3.2 Effects of transport duration

(i) ***Rectal temperature and temperature humidity index during transport***

The mean rectal temperature profiles and the temperature and humidity index (THI) on board the vehicles during the transport phase are shown in Figure 4.5a,b. The rectal temperatures and THI data shown are an average for the two replicates for each transport duration treatment. There were similar trends observed during the initial stages of transport. There was a rise in rectal temperature at loading and the initial 60-120 minutes of the journey followed by a decline in temperature. Over the longer journeys there were slight fluctuations in rectal temperature, which are attributed to diurnal changes. The rectal temperatures recorded in this study stayed within the normal range for cattle of 37.5-39.5 ^{o}C (Ruckebusch et al., 1991).



a.

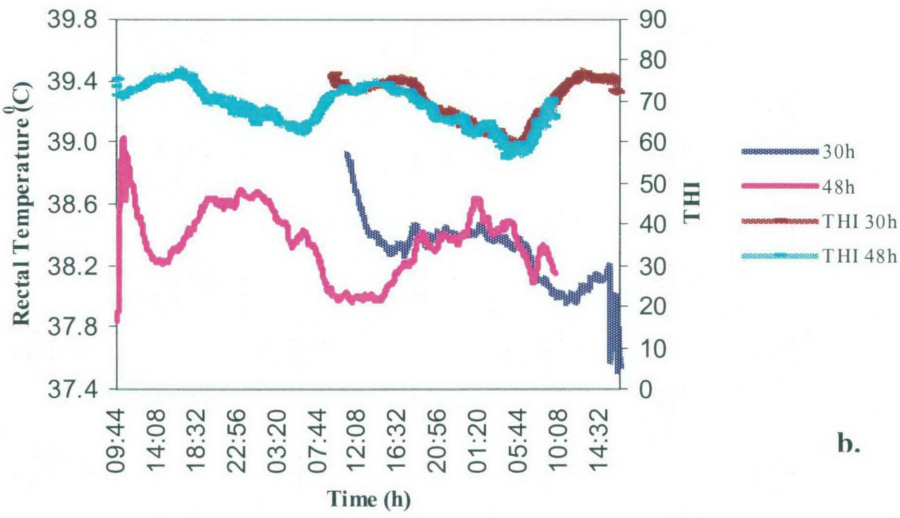


Figure 4.5 Rectal temperature and temperature and humidity index recorded over (a) 6 and 12 h of transport and (b) 30 and 48 h of transport

There was very little variation in the mean daily rectal temperatures during the 72 h of recovery, as illustrated in Table 4.6. There was no elevation of rectal temperature as a result of transport treatment.

Table 4.6 Mean rectal temperatures (C^{o}) during recovery from 6, 12, 30 and 48 h transport treatments

Transport duration (h)	Arrival	24h	72h
6	38.51	38.30	38.31
12	38.64	38.17	37.79
30	38.28	37.58	37.62
48	38.36	38.36	38.36

In addition to the main effects, the interaction between transport duration × sampling × replicate was significant for BUN, TP, BHB, OSMOL, liveweight, HGB, HCT, MCV, MCHC, NEU, LYM and N:L ratio. This was largely due to the difference between replicates. Notwithstanding this, emphasis was given in the following results to the interaction between transport treatment × sampling time as this was the main focus of the experiment.

(ii) ***Liveweight***

A significant ($P<0.01$) transport duration × replicate × sampling time interaction was observed for liveweight (Table 4.7) along with significant interactions for replicate × sampling time ($P<0.001$) and transport duration × sampling time ($P<0.001$). There was a direct relationship between transport duration and liveweight loss ($P<0.001$) (Figure 4.6). For the interaction transport duration × sampling time, the significant difference between treatments was only evident on arrival where the liveweight for the 48 h treatment group was significantly lower ($P<0.01$) than the other duration treatments (Figure 4.6). The mean weight loss for each treatment was: 5.4%, 6.0%, 6.8% and 10.2% for the 6, 12, 30 and 48 h transport treatments, respectively. By the end of the 72 h recovery period, the cattle had recovered 95-98% of their pre-transport liveweight.

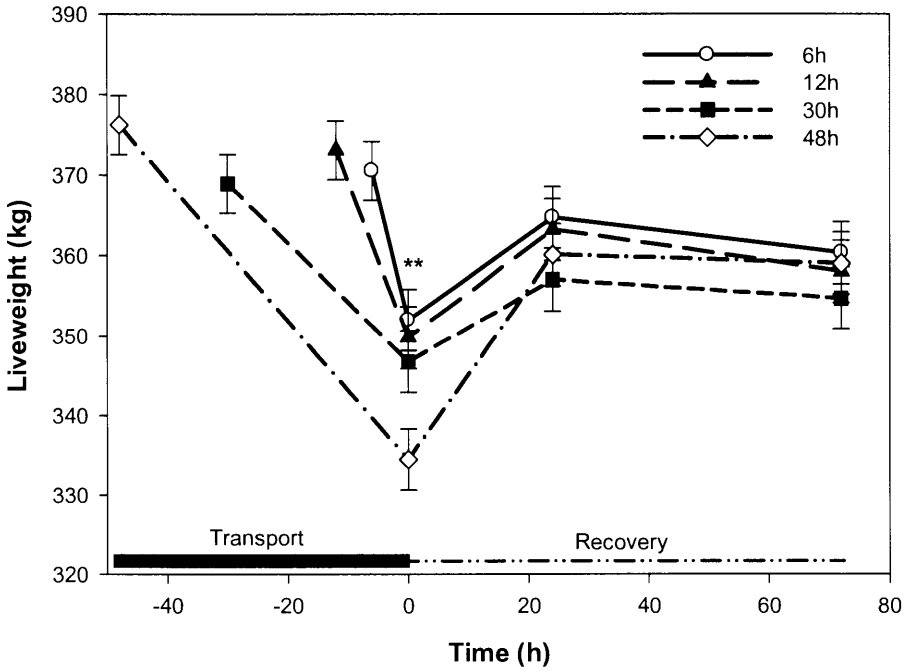


Figure 4.6 Liveweight changes due to transport for 6, 12, 30 and 48 h and during 72 h of recovery (***, ** and * Means at each time point are significantly different at the P<0.001, P<0.01, P<0.05 levels, respectively)

(iii) ***Blood chemistry***

The statistical significance of the main effects and their interaction are shown in Tables 4.7 and 4.8 for blood chemistry (BUN, CK, TP, ALB, BHB, cortisol, OSMOL, HAPT), and haematology (HGB, RBC, HCT, MCV, MCH, MCHC, PCV, WBC, NEU, LYM, MON, EOS, BAS and N:L ratio) measures, respectively.

Blood urea nitrogen and β-hydroxybutyrate

Significant interactions between transport duration × replicate × sampling time (P<0.001), replicate × sampling time (P<0.001), transport duration × sampling time (P<0.001) and

transport duration × replicate ($P<0.05$) were observed for BUN (Table 4.7). For the interaction transport duration × sampling time there were significant differences in BUN levels between the transport duration treatments at all three post-transport sampling times (0, 24 and 72 h) (Figure 4.7). Transport elicited an increase in BUN and the highest levels were observed after the longer journeys. BUN rapidly decreased during the first 24 hours post transport and the concentrations had returned to pre-transport values within the first 24 h of recovery (Figure 4.7). After the 72 h recovery period, BUN concentrations were below the pre-transport values.

Significant interactions between transport duration × replicate × sampling time ($P<0.01$), transport duration × sampling time ($P<0.01$) were observed for BHB. There were significant differences in BHB levels between the transport duration treatments at all three post-transport sampling times (0, 24 and 72 h) (Figure 4.7). The blood concentrations of BHB decreased (Figure 4.7) in all four treatment groups at the conclusion of the transport. There was a trend for these to increase during the recovery phase, with the exception of the 12 h treatment group (Figure 4.7).

Creatine kinase

There were no significant interactions observed for CK, however, there was a significant effect of sampling time ($P<0.001$). There were increases in CK following transport however the differences between treatments were not significant. The greatest increases in CK were observed in the 48 h treatment group (Figure 4.7). There was a significant

($P<0.001$) decline in CK levels after the initial 24 h of recovery (43%) and this trend continued during the remainder of the recovery phase (Figure 4.7).

Cortisol

A significant transport duration × sampling time ($P<0.001$) interaction was observed for cortisol. The levels of cortisol were lower at arrival than when transport commenced for all four treatments. For the interaction transport duration × sampling time, the significant difference between treatments was only evident on arrival where the cortisol concentration for the 48 h treatment group was significantly higher ($P<0.001$) than the other duration treatments (Figure 4.7).

Total protein, albumin, haematocrit and osmolality

The trends for these indicators of haemoconcentration and therefore dehydration were very similar. Transport resulted in haemoconcentration and it tended to be higher, the longer the journey. During recovery when the animals had access to water, the levels rapidly returned to, at or near their pre-transport levels.

There were interactions observed between transport duration × replicate × sampling time ($P<0.01$) for TP and a replicate × sampling time ($P<0.001$) and transport duration × sampling time ($P<0.001$ and $P<0.01$) for TP and ALB, respectively.

The concentration of TP and ALB increased with transport where the largest changes occurred in the journeys of longer duration. Significant treatment differences were only

evident on arrival for both variables. The 6 and 12 h treatment groups were significantly lower than the transport treatments of 30 and 48 h. However, significant recovery had been achieved after 24 h post transport (Figure 4.8).

For haematocrit the interactions of transport duration × replicate × sampling time ($P<0.001$) and transport duration × sampling time ($P<0.001$) and replicate × sampling time ($P<0.05$) were significant. There were significant differences between the four transport duration treatments on arrival ($P<0.01$), where the 6 and 48 h treatments were significantly higher than the 12 and 30 h. During recovery the 6 h treatment group was different to 12, 30 and 48 h. The change in haematocrit during recovery ranged from 1-2% (Table 4.5 and 4.8).

A number of significant interactions were found for osmolality including, transport duration × sampling time × replicate ($P<0.001$), transport duration × replicate ($P<0.01$), transport duration × sampling time ($P<0.001$), and replicate × sampling time ($P<0.001$). Significant treatment differences were found at arrival and at each post transport time point (Figure 4.8). On arrival the 30 and 48 h treatments were significantly higher than the 6 and 12 h transport treatments. After 24 h of recovery, the 12 and 48 h treatments were different to the 6 and 30 h treatments. At the end of the recovery period the 6 h treatment was significantly higher than the other three duration treatment groups.

Table 4.7 Effect of transport duration, replicate and sampling time and their interactions on the changes in liveweight and serum levels of BUN, CK, TP, ALB, BHB, cortisol, OSMOL. and HAPT.

	BUN	CK†	TP	ALB†	BHB†	Cortisol†	Osmol.	Hapt.†	Liveweight
Main Effects									
Transport Duration (h)									
6	12.25	265.2	73.51	38.1	0.17	76.8	295.9	1.05	358.99
12	12.52	326.04	73.85	37.57	0.16	79.8	297	1.09	357
30	12.61	373.97	74.41	38.44	0.12	72.4	297.71	1.1	352.83
48	13.11	397.2	74.01	38.32	0.17	86.2	298.27	1.13	351.38
SED	0.453	0.0057	1.03	0.01	0.02	0.44	1.26	0.03	5.21
Significance	ns	ns	ns	ns	**	ns	ns	ns	ns
Replicate									
1	11.76	334.94	73.72	38.6	0.17	91.4	297.28	1.1	355.26
2	13.49	336.08	74.17	37.8	0.14	67.04	297.15	1.09	354.84
SED	0.32	0.004	0.73	0.01	0.01	0.31	0.89	0.02	3.67
Significance	***	ns	ns	*	**	***	ns	ns	ns
Sampling Time									
0	16.34	695.4	76.05	39.34	0.16	72	301.12	1.02	345.78
24	10.51	393	72.43	37.6	0.16	84.1	297.81	1.09	361.38
72	11.02	165.6	73.37	37.7	0.15	80.5	292.72	1.17	358
SED	0.31	0.0016	0.52	0.01	0.05	0.2	0.91	0.03	0.98
Significance	***	***	***	***	ns	**	***	***	***
Interaction									
Transport Duration x Replicate	*	ns	ns	ns	ns	ns	**	ns	ns
Transport Duration x Sampling Time	***	ns	***	**	**	***	***	ns	***
Replicate x Sampling Time	***	ns	***	***	ns	ns	***	ns	***
Transport Duration x Replicate x Sampling Time	***	ns	**	ns	**	ns	***	ns	**

†Back transformed means shown, *** P<0.001, ** P<0.01, * P<0.05; ns – not significant; SED – Standard error of the difference BUN (mg/dL), CK (U/L), TP (g/dL), ALB (g/L), BHB (mmol/L), serum concentrations of cortisol (nmol/L), OSMOL (mOsmol/L), HAPT (mg/dL) and liveweight (kg).

Table 4.8 Effect of transport duration, replicate, sampling time and their interaction on the changes in haematology measures.

	HGB	RBC†	HCT	MCV	MCH	MCHC	PCV	WBC†	NEU†	LYM†	MON†	EOS†	BAS†	N:L† Ratio
Main Effects														
Transport Duration (h)														
6	148.58	10.35	43.9	46.98	16.02	339.56	455.38	10	4.92	3.82	1.72	1.35	1.03	2.54
12	143.88	10.19	41.86	45.62	15.78	345.26	451.44	8.5	5.11	3.17	1.57	1.24	1.04	3.06
30	144.18	10	42.23	47.01	16.08	341.47	485.54	8.51	3.49	4.49	1.57	1.38	1.07	1.86
48	145.81	10.03	42.82	47.58	16.2	340.86	426.34	7.27	3.78	3.12	1.59	1.37	1.05	2.51
SED	2.6	0.025	0.008	1.02	0.36	1.47	35.96	0.05	0.08	0.07	0.06	0.04	0.007	0.08
Significance	ns	ns	*	ns	ns	***	ns	***	***	***	ns	*	***	***
Replicate														
1	144.89	10.08	41.18	45.46	16.05	352.15	426.34	8.69	4.21	3.83	1.74	1.35	1.04	2.31
2	146.33	10.2	44.22	48.14	15.99	331.42	486.26	8.35	4.33	3.5	1.49	1.32	1.06	2.61
SED	1.84	0.018	0.005	0.72	0.26	1.04	25.42	0.03	0.05	0.05	0.04	0.03	0.005	0.08
Significance	ns	ns	***	***	ns	***	*	ns	ns	ns	***	ns	***	*
Sampling Time														
0	150.29	10.52	43.57	45.82	15.86	345.94	453.92	10.78	6.49	3.72	1.71	1.13	1.04	3.4
24	141.99	9.89	41.38	46.63	16.12	343.85	468.03	8.43	3.92	3.81	1.54	1.7	1.04	2.22
72	144.55	10.03	43.15	47.93	16.08	335.57	446.94	6.8	3.05	3.47	1.59	1.24	1.07	1.97
SED	0.57	0.004	0.002	0.09	0.048	0.84	14.4	0.02	0.03	0.03	0.03	0.02	0.006	0.03
Significance	***	***	***	***	***	***	ns	***	***	**	**	***	***	***
Interaction														
Treatment x Replicate	ns	ns	ns	ns	ns	***	**	ns	*	ns	**	ns	ns	**
Treatment x Sampling Time	***	***	***	***	ns	***	*	***	***	***	***	***	***	***
Replicate x Sampling Time	***	*	*	ns	***	***	***	***	***	***	*	**	ns	***
Transport Duration x Replicate x Sampling Time	***	ns	***	***	ns	***	ns	ns	***	**	ns	ns	ns	***

†Back transformed means shown, *** P<0.001, ** P<0.01, * P<0.05; ns – Not significant; SED – Standard error of the difference
HGB (g/dL), RBC (x 10^{12}/L), HCT %, MCV (x$1e^{-15}$/L), MCH (x$1e^{-12}$), MCHC (g/L), PCV (µ/L), WBC (x 10^{9}/L), NEU (x 10^{9}/L), LYM (x 10^{9}/L), MON (x 10^{9}/L), EOS (x 10^{9}/L), BAS (x 10^{9}/L) and N:L Ratio

Table 4.9 Least square means for the interaction transport duration × sampling time for haematology measures

Variable	Time (h) Post Transport	Journey Duration			
		6 h	12 h	30 h	48 h
HGB	0	150.5	148.2	149.1	153.4
	24	$145.8^{a,c}$	$142^{b,c}$	$139.6^{b,c,d}$	$140.5^{a,d}$
	72	149.5^{a}	141.4^{b}	143.9^{b}	143.5^{b}
RBC†	0	10.5	10.6	10.4	10.6
	24	$10.2^{a,c}$	10^{a}	9.7^{b}	9.7^{b}
	72	10.3	10	9.9	10
MCV	0	46.1^{a}	$44.6^{a,c}$	45.6^{a}	$47^{a,b}$
	24	46.8^{a}	$45.5^{a,c}$	46.7^{a}	$47.6^{a,b}$
	72	48.1	46.8	48.7	48.1
MCH	0	15.9	15.6	15.9	16.0
	24	16.0	15.9	16.2	16.3
	72	16.1	15.8	16.1	16.3
MCHC	0	344.3^{a}	348.4^{b}	349.2^{b}	341.8^{a}
	24	341.1^{a}	348.6^{b}	$344.2^{a,c}$	$341.5^{a,c}$
	72	333.2^{a}	338.8^{b}	331^{a}	339.3^{b}
PCV	0	475.9^{a}	416.7^{b}	499.3^{a}	423.8^{b}
	24	450.3^{a}	500.1^{b}	501.5^{b}	420.2^{a}
	72	440	437.5	455.8	454.5
NEU†	0	9.8^{a}	7.7^{b}	4.4^{c}	5.4^{d}
	24	4^{a}	$4.6^{a,c}$	$3.6^{a,b}$	$3.5^{a,b}$
	72	3^{a}	3.8^{b}	2.7^{a}	2.8^{a}
LYM†	0	2^{a}	1.7^{a}	1.4^{b}	1.8^{a}
	24	1.6^{a}	1.4^{b}	1.7^{a}	1.4^{b}
	72	1.6^{a}	1.7^{b}	1.6^{a}	1.5^{b}
MON†	0	2^{a}	1.7^{b}	1.4^{c}	$1.8^{a,b}$
	24	1.6^{a}	$1.4^{a,c}$	$1.7^{a,b,d}$	$1.5^{a,d}$
	72	1.6	1.7	1.6	1.5
EOS†	0	1.1^{a}	1^{a}	1.2^{b}	1.2^{b}
	24	1.8^{a}	1.5^{b}	1.8^{a}	1.8^{a}
	72	1.3	1.2	1.2	1.2
BAS†	0	1^{a}	1.04^{b}	1.07^{c}	1.06^{c}
	24	1.04	1.03	1.05	1.03
	72	1.06^{a}	1.07^{a}	1.1^{b}	1.06^{a}

†Back transformed means shown

$^{a, b, c, d, e}$ Means at each time without a common superscript differ (P<0.0.5)

HGB (g/dL), RBC (x 10^{12}/L), MCV (x $1e^{-15}$/L), MCH (x $1e^{-12}$), MCHC (g/L), PCV (µ/L), NEU (x 10^{9}/L), LYM (x 10^{9}/L), MON (x 10^{9}/L), EOS (x 10^{9}/L) and BAS (x 10^{9}/L)

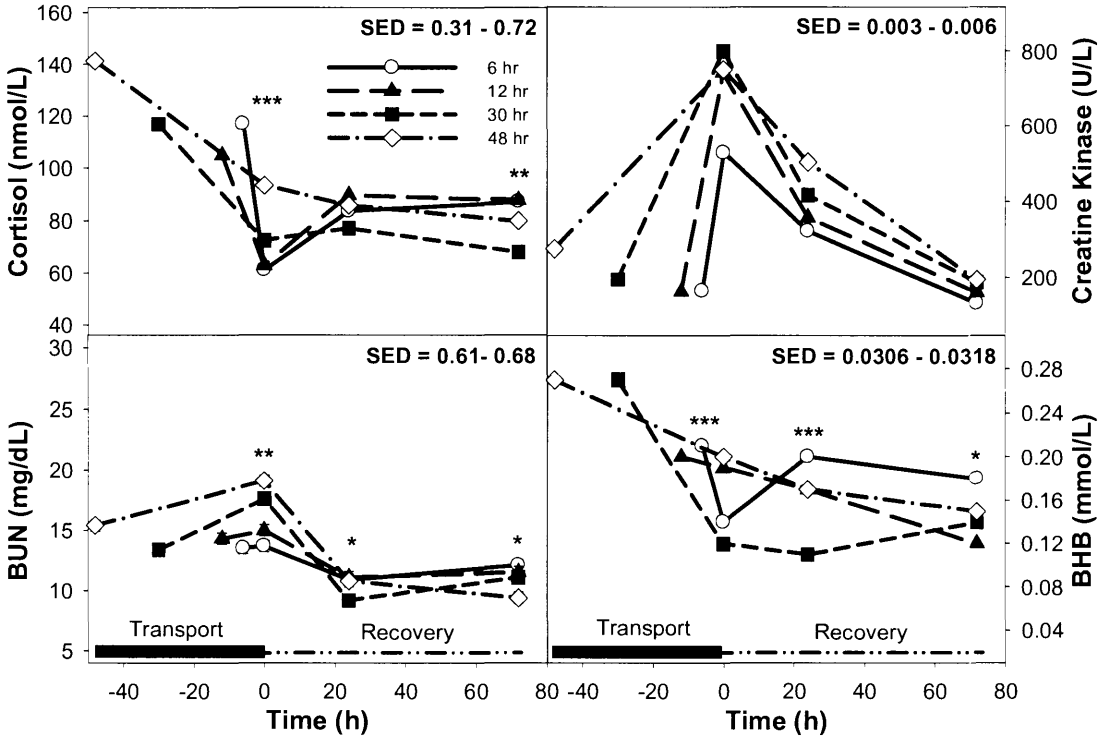


Figure 4.7 Least square means for the effect of transport duration × sampling time on plasma levels of cortisol, CK, BUN and BHB. (***, ** and * Means at each time point are significantly different at the P<0.001, P<0.01 and P<0.05 levels, respectively)

Haptoglobin

There were no significant interactions for haptoglobin, however there was a significant sampling time effect (P<0.001) where HAPT increased over the recovery phase (Figure 4.9). The concentration of haptoglobin recorded at 72 h post transport was some 20% higher than that observed prior to transport.

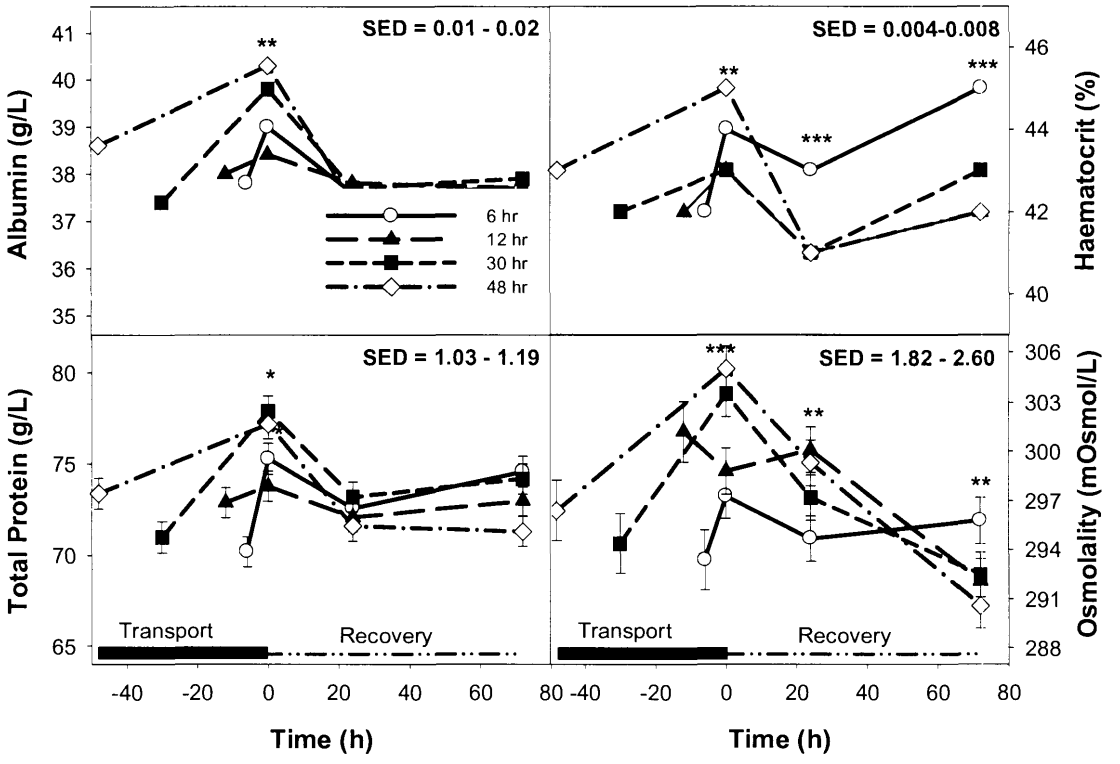


Figure 4.8 Least square means for the effects of transport duration × sampling time on ALB and TP concentration, haematocrit, and osmolality. (***, ** and * Means at each time point are significantly different at the P<0.001, P<0.01 and P<0.05 levels, respectively)

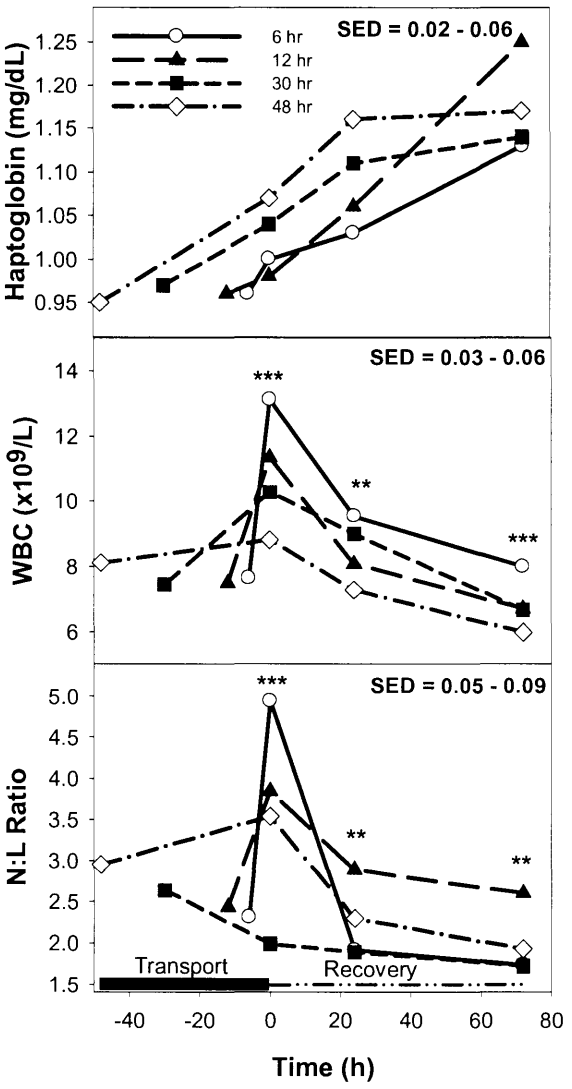


Figure 4.9 Least square means for the effects of transport duration × time on haptoglobin concentration, white blood cell count and Neutrophil:Lymphocyte (N:L) ratio. (***, ** and * Means at each time point are significantly different at the $P<0.001$, $P<0.01$ and $P<0.05$ levels, respectively)

(iv) ***Haematology***

The interaction transport duration × sampling time was significant for all the haematology measures with the exception of MCH (Table 4.8). Of these, emphasis was given to the changes in WBC, N:L and RBC as these are commonly affected by stress.

White blood cell count

Transport duration × sampling time ($P<0.001$) and replicate × sampling ($P<0.001$) interactions were significant for white blood cell count. The highest WBC upon arrival was observed for the 6 h treatment. The highest white cell counts were recorded at the end of the transport phase, 71% increase for 6 h, 51% increase for 12 h, 38% increase for 30 h and a 9% increase for the 48 h treatment group. The WBC counts at 24 and 72 h post-transport continued to decline during the recovery phase and were close to, or at pre-transport levels after 72 hours of recovery. For the interaction transport duration × sampling time, the significant difference between treatments was evident on arrival, and during recovery at 24 and 72 h. There were differences between the 6 and 48 h treatments compared to the 12 and 30 h treatments at arrival. During recovery the 6 h treatment group was different to 12, 30 and 48 h

N:L Ratio

Significant transport duration × replicate × sampling time ($P<0.001$), transport duration × sampling time ($P<0.001$), transport duration × replicate ($P<0.01$) and replicate × sampling time ($P<0.001$) interactions were observed for N:L ratio. Similarly with N:L ratio, the highest ratio was observed for 6 h transport duration on arrival (Figure 4.9). For the

interaction transport duration × sampling time, there was significant differences between treatments, where the 6 h treatment was higher ($P<0.001$) than the other duration treatments. The 6 h treatment remained significantly higher than the 12, 30 and 48 h treatments during recovery also. Recovery rates for all transport groups (Table 4.6) had returned to, or close to pre-transport levels after 72 hours of recovery (Figure 4.9).

Red blood cell count

Transport duration × sampling time ($P<0.001$) and replicate × sampling ($P<0.05$) interactions were significant for red blood cell count. The greatest increases in RBC upon arrival were observed in the short duration treatment of 6 h. For the interaction transport duration × sampling time, significant difference between treatments was only evident 24 h into recovery where the RBC for 30 and 48 h treatment groups was significantly lower than 6 and 12 h duration treatments (Table 4.9). The RBC counts at 24 and 72 h post-transport continued to decline during the recovery phase and were close to pre-transport levels after 72 hours of recovery.

(v) ***Behaviour***

Specific behaviours including the time spent lying and eating and number of drinking bouts were assessed on the 12 and 48 h transport duration groups during the first 6 hours of recovery.

Figure 4.10 Cattle in the initial period (1 h) of recovery. The 48 h transport duration group are in the near pen and the 12 h transport duration group in the far pen

The results for the time spent lying for each replicate are presented in Table 4.9. There was a significant ($P<0.001$) difference in lying time between the two treatment groups, where the 48 h treatment group were lying more, particularly during the first 3 hours of recovery. The results were less clear during the second 3 h of recovery as the results differed between replicates. It was also pertinent to note that for the second replicate in the later part of the afternoon, 4 hours into the recovery period, it rained heavily, and this may have contributed to the difference between replicates.

Table 4.10 The time (minutes) spent lying and eating and the number of drinking bouts for the 12 and 48 h transport groups during the initial 6 h of recovery

Behaviour during post-transport recovery	Replicate 1			Replicate 2		
	12 h	**48 h**	**Significance**	**12 h**	**48 h**	**Significance**
Lying time						
0 – 3 h	29	132	P<0.001	36	139	P<0.001
3 – 6 h	74	77	*ns*	38	89	P<0.001
Eating time						
0 – 3 h	16	6	P<0.05	19	8	P<0.05
3 – 6 h	12	27	P<0.05	22	28	*ns*
Drinking bouts						
0 – 3 h	26	21	~	97	9	~
3 – 6 h	25	41		21	13	

There was a significant difference in the time spent eating during the first 3 hours post arrival in both replicates (Table 4.9). The animals that had been transported for 48 h had less desire to eat as they spent more time lying.

Similarly, with the drinking bouts, it was apparent that whilst animals in the 48 h treatment group had been deprived of water for 48 hours, their behaviour reflected a stronger desire to rest rather than drink (table 4.9). It was not possible to measure water intake.

(vi) ***Feedlot average daily gain (ADG)***

The ADG after 42 days in the feedlot did not significantly differ between the transport duration treatments. The ADG least square means for each treatment was: 1.8, 2.1, 2.08 and 1.93 kg/day for the 6, 12, 30 and 48 h transport durations, respectively.

4.4 Discussion

The purpose of this study was to quantify the physiological and behavioural responses of *Bos indicus* x *Bos taurus* heifers transported for journey durations of 6, 12, 30 and 48 h. The main findings of this study were that the transported animals lost between 5.4 and 10.2% of their liveweight during the transport phase. The changes in blood chemistry and haematology measures did indicate some level of dehydration, tissue catabolism, muscle activity/damage and immune cell changes due to transport. However, it is important to note that the majority of these transport-mediated changes, when compared with normal clinical values for cattle, were still within the normal ranges (eg. Kaneko et al., 1997). Furthermore, for the majority of these measures they had returned very close to, at or below their pre-transport levels after 72 h of recovery. There was no sustained effect of transport on animal productivity or health during feedlot finishing.

The losses in liveweight observed here were similar to those recorded in other cattle transport studies (Tarrant, 1990; Warriss et al., 1995; Knowles et al., 1999) where weight loss increased with journey length and time off feed and water. The trend wasn't linear as it is generally recognised that the largest losses in weight, predominantly gut fill, occur in the first 12 h of food and water deprivation (Wythes, 1987). Phillips et al. (1991) showed that 61-64% of the total liveweight loss after 48 hours of food and water deprivation came from excreta. An important outcome of the present study was that the cattle had successfully recovered 95-98% of their weight loss after 72 h of recovery with adequate amounts of rest, free access to good quality water and forage hay. In two other cattle transport studies, where steers were transported for durations ranging 5 - 18 h, the weight

loss ranged from 3-11% and it took 4-5 days for the cattle to recover this weight (Phillips et al., 1991; Warriss et al., 1995). Warriss et al. (1995) did not state whether the animals were fasted prior to transport, however, in the Phillips et al. (1991) study, steers were fasted for a total period of 48 h and for 18 h of this, the cattle were transported.

The THI for all four journey durations were within the acceptable range. As reported by Silanikove (2000), THI values between 75-80 represent mild thermal stress, while values ≥80 indicate moderate to severe stress. In this study, during the course of the journeys, the THI did not exceed values higher than 80 and there were only short periods during the journey where the THI ranged between 75-80. Therefore at worst, the cattle may have experienced short periods of mild thermal discomfort.

There were elevated levels of cortisol at the start of transport for all four treatment groups, when compared with normal blood analyte values in cattle (Kaneko et al., 1997). This elevation in cortisol was most likely associated with the movement and handling prior to transport. Cortisol concentrations for the 48 h treatment group were notably high at the commencement of transport at 141.40 nmol/L. Given that the 48 h treatment group was the first scheduled departure it is possible there may have been some carry-over effects from the day before when the cattle were weighed and allocated to their treatments groups. The concentrations of serum cortisol were lower on arrival at the feedlot when compared with those at departure. Relative to the pre-transport sample where there was a pronounced HPA response due to handling, the decrease in cortisol concentrations after transport, irrespective of duration could be interpreted as the cattle were less stressed. The changes in

rectal temperature indicate there was a stress-mediated rise in rectal temperature during loading and the first two hours of the journey followed by a decline in temperature. This data and the temperature and cortisol results from Chapter 3 generally support the view that the initial acute physiological response at commencement of transport dissipates as the cattle habituate to the transport conditions. Similar results were observed by Warriss et al. (1995) in their study where cattle were transported for 5, 10 and 15 hours. Parrott et al. (1998) also reported that cattle eventually appear to adapt to motion stimuli during extended periods of transport. Although additional cortisol samples were not collected during the actual transport phase, it is reasonable to assume given these results, that the cattle have habituated to transport and this probably accounts for the lower cortisol response after transport. The cortisol levels at 24 and 72 h of recovery were at a level indicative of some HPA activation. This is probably not a carry-over effect of the transport rather it is probably due to handling the cattle received during movement from their pens and into the yards prior to blood sampling.

There were increased levels of CK at the conclusion of the transport phase although the differences between treatments were not significant. An increase in CK levels is indicative of increased muscle activity and damage which was expected when considering the physical demands associated with handling and transport (Tarrant and Grandin, 2000). Standing and maintaining balance during transport requires varying levels of muscle exertion, therefore muscle fatigue may become a factor particularly over long durations when animals cannot lay down (Knowles et al., 1999; Swanson and Morrow-Tesch, 2001). In a study by Knowles et al. (1999), CK levels also increased over 31 hours of transport. In

the present study, there was very little difference in the immediate post-transport CK levels particularly between the transport durations of 12, 30 and 48 h. The CK concentrations 24 hours into recovery had declined considerably, and by 72 hours post transport, all groups had returned to pre-transport levels indicating that there was not a sustained effect on muscle integrity. The recovery rates are similar to those found in two other cattle transport studies (Warriss et al., 1995; Knowles et al., 1999) where it took between 2-5 days post-transport before the CK levels had returned to their pre-transport levels.

It would be expected that deprivation of food and water for up to 48 h would bring about some metabolic changes in cattle. Therefore, in this study it was important to assess parameters that may be indicative of altered metabolic status and the blood concentrations of BUN and BHB are useful measures (Knowles et al., 1993, 1994; Shorthose and Wythes, 1988). The increase in BUN due to transport did not exceed the normal upper level of (20-30 mg/dL) reported for mature healthy cattle (Kaneko et al., 1997). This moderate increase in BUN perhaps indicates some protein catabolism, but this rise may have also been due to the disruption of the animal's normal pattern of eating (Warriss et al., 1995). This suggests that the period of fasting imposed during transport did not result in a major increase in protein catabolism.

The decrease in serum BHB levels following transport was consistent with the findings of Knowles et al. (1999) who found a slight but significant decrease for transport durations of up to 31 h. These results appear counter-intuitive and a possible reason for the same result in the present study is that the animals were not subjected to a fasting period prior to

transport. Knowles et al. (1999) suggested that the BHB decrease may have been due to the combined effects of the journey and change in diet, when animals were adapting to being fed hay after grazing on grass.

In another transport study (Earley et al., 2004) where cattle were fasted and then transported, they concluded that the combination of 8 h of fasting and 8 h of transport did not negatively impact upon animal welfare. Whilst BHB and BUN increased it was not significant and there were minimal differences in the treatments after 8 h of fasting (with access to water) versus no fasting on the responses to 8 h of road transport. Similarly in the study by Phillips et al. (1991), there were no differences in the BUN levels for cattle that were restricted of food and water for 48 h and transported for 18 h during this 48 h period when compared to the non fasted controls.

The measures that indicate haemoconcentration (haematocrit, serum total protein, albumin and osmolality), and therefore hydration status, all exhibited similar changes in response to transport duration. These variables increased commensurate with transport duration indicating that journey duration did impact progressively on the hydration status of the cattle. However, the levels were generally still within or close to normal clinical ranges (Boyd, 1984; Kaneko et a., 1997). On arrival, the serum albumin concentrations were between 35 – 40 g/L for the transport duration treatments which is marginally above the normal upper limit for cattle (Boyd, 1984; Kaneko et al., 1997). The 6 and 12 h transport durations for total protein were below the levels of the 30 and 48 h transport duration groups, which had the highest values of 77.9 and 77.2 g/L respectively. The 30 and 48 h

transport groups were only just below the normal upper limit of approximately 81 g/L (Boyd, 1984), therefore, it is evident from these results that the journeys of 30 and 48 h were impacting upon the hydration status of the cattle. The serum measure of osmolality is quite informative in the context of dehydration (Broom et al., 1996). On arrival, the osmolality levels were at their greatest for 30 and 48 h treatment groups.

Examining the results from cattle transport studies, Warriss et al. (1995) reported evidence of slight dehydration after transport for 10 and 15 h, as did Knowles et al. (1999) for durations up to 31 h based on changes in osmolality, total protein and albumin. In the 31 h transport group of the Knowles et al. (1999) study, total protein increased from 79 to 87 g/L and the concentration of albumin ranged from 39.8 to 43.1g/L. Simialr elevations in these parameters were found in the present experiment particularly for the 30 and 48 h transport durations.

There were also transport-mediated increases in haematocrit. This effect was also observed in a study of long distance transport (2200 km) by Fazio et al. (2005) and other cattle transport studies (Atkinson, 1992; Tarrant et al., 1997; Schaefer et al., 1997). Haematocrit can be the least informative measure of hydration status as it can be affected by splenic contraction of red blood cells, as well as dehydration, and caution needs to be taken when interpreting measures of haematocrit (Knowles and Warriss, 2000).

In the present experiment it is important to note that the cattle very quickly rehydrated with access to water. A substantial recovery was achieved within the first 24 h post-transport and a complete recovery had occurred at 72 h.

The stress associated with handling, loading and transport has been shown to elicit increases in white blood cell counts which are largely due to neutrophilia (Swanson and Morrow-Tesch, 2001; Tarrant et al., 1992). This was also evident in the present study but mainly for the shorter journey durations of 6 and 12 h. It is likely that this is related to the physiological response seen during the initial stages of transport (Chapter 3), and that the same changes occurred in the animals on the longer duration treatments, but the increase was resolved by the time of post-transport sampling. Along with neutrophilia, lymphopenia has also been observed with transport (Murata et al., 1987; Phillips et al., 1989) and therefore the ratio of neutrophils:lymphocytes can be a very informative measure of stress mediated changes in immune cell numbers. While in the present study there was a significant transport duration × sampling time interaction for N:L ratio, in general the haematology results did not indicate a significant degree of compromise associated with transport. Although there was a greater decline in red cell counts for animals transported for 30 and 48 h, the decrease was not of clinical significance. Almost all the blood chemistry and haematology measures in this study had returned to pre-transport levels after 72 h of recovery.

After a transport-mediated increase, the serum haptoglobin continued to increase during recovery. Haptoglobin levels in blood can remain elevated for over 4 days following a

short period of activation (Peppard et al., 1994). Therefore haptoglobin can be a valuable marker of physiological responses (Peppard et al., 1994) as it mops up excess haemoglobin that is circulating in the blood as a result of tissue injury and inflammation (Murata and Miyamoto, 1993). In the present study, the haptoglobin levels for 30 and 48 h treatment groups were beginning to plateau by 72 hours after transport, suggesting that there was not a sustained long term activation of this particular acute phase protein. Murata and Miyamoto (1993) reported increased haptoglobin levels after transporting 6 month old calves for 1400 km over 2 days in which they were fed roughage and water at three stops. This elevation in haptoglobin is indicative of an impact on homeostatic mechanisms that can influence subsequent immune responses (Eicher, 2001). The clearance of the haptoglobin protein is slow, and as reported above may take as long as four days to be removed from the body after secretion. Furthermore, because the changes in immune cell counts had returned to normal, the sustained increase in haptoglobin was not a major cause for concern.

It was evident that cattle transported over the longer durations of 30 and 48 h were more fatigued on arrival, based on behavioural observations. During the first 6 h of recovery the 48 h treatment group exhibited increased lying behaviour particularly during the first 3 h of recovery compared to the 12 h group. Increased lying behaviour after arrival has also been observed in other cattle transport studies after unloading, in both long (24 h) (Tarrant et al., 1992) and also short (1 h) haul transport by Kenny and Tarrant, (1987[ab]). Knowles et al. (1999) reported some cattle started to lie down in transit after 20 h of transport. They attributed this was because animals were becoming fatigued. However in the present

experiment, the effects of transportation for long periods was relatively short-lived as the differences in the second three hour period were less clear in that the cattle from the 48 h transport group had started to exhibit comparable behaviours to the 12 h treatment group.

The number of drinking bouts and the time spent eating that occurred in the early stages of the recovery period were minimal for the 48 h treatment in comparison to the 12 h treatment group, particularly in the first three hours of recovery. However, it is important to note that while the number of drinking bouts was less for the 48 h group, it was not possible to quantify the amount of water consumed/bout. It is possible that this may have been higher for the 48 h group. Despite the fact that the 48 h treatment group had no feed or water for that period, their strongest desire was to rest, especially in the first three hours of recovery. Once they had a 3-hour rest period, drinking and eating behaviour gradually increased.

4.5 Conclusion

This is the first study undertaken in Australia investigating the physiological and behavioural responses in cattle following road transport durations up to 48 h. The blood variables measured in this experiment did reflect signs of mild dehydration, tissue catabolism and muscle exertion, though these physiological measures were still within normal clinical ranges. However, after 72 h of recovery with access to fresh clean water and good quality forage hay all the variables had returned to, or close to their pre-transport levels. The results suggest that healthy cattle that have not had restricted access to food or

water prior to transport and transported in accordance with best practice, can cope with transport durations up to 48 h without major compromise to their welfare.

Chapter 5

5. GENERAL CONCLUSIONS

The primary aim of this research was to investigate under Australian conditions, how cattle respond and cope with differing loading treatments, journey durations and how quickly they recover post transport. The research reported in this thesis has provided a better understanding of how cattle respond to the stressors that apply during transport under Australian conditions.

The effects of different loading practices were examined in the first experiment. It was concluded that cattle subjected to the appropriate use of an electric prodder during loading did not exhibit significantly different physiological responses to those that were loaded onto the truck without the use of a prodder. It is possible that the specific effect of the electric prodder *per se* may have been overridden by the significant physiological response that occurred during loading and handling. It is important to emphasise that this conclusion should not be interpreted as an endorsement for prodders at loading. Excessive use of prodders at loading may quickly alter animal behaviour, which in turn, makes some animals more agitated and difficult to handle and in some situations animal welfare may be compromised. If the use of such devices is necessary, their use should be kept to an absolute minimum and used appropriately as advocated by Grandin (1997).

From the second major experiment quantifying the impact of transport duration, the results indicate that whilst there was a biological cost to the animal, they were not presented with a physiological challenge that was beyond their ability to cope. It was concluded that

healthy cattle that don't have a food or water restriction prior to transport are able to cope with transport for durations up to 48 hours without compromise to their welfare. Moreover, with adequate food and rest they were able to recover within 72 hours indicating best practice transport of up to 48 hours is acceptable from a welfare standpoint. However, in making this conclusion, it must be kept in context, in that they can not be extrapolated across all long-haul transport events, as the condition of livestock and the transport conditions (e.g. weather) will potentially influence the capacity of the animals to cope with transport stress. Thus while the Model Code of Practice for Land Transport of Cattle provides for maximum limits on journey duration, it continues to be the responsibility of those in charge of livestock to manage the animals in a way that results in acceptable animal welfare.

The impact of land transport upon different classes of cattle, under extreme environmental conditions with different curfew treatments and transport durations cannot be extrapolated from the results of this thesis. Further experimental work is required to expand the scope of this research and quantify welfare outcomes across differing stock classes and transport conditions.

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