finalreport

Animal Welfare

Project code: **AHW.055**
B.AHW.0055
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Date published: **May 2008**
ISBN: **9781741912470**

PUBLISHED BY
Meat & Livestock Australia Limited
Locked Bag 991
NORTH SYDNEY NSW 2059

Animal Welfare Outcomes of Livestock Road Transport Practices

Meat & Livestock Australia acknowledges the matching funds provided by the Australian Government to support the research and development detailed in this publication.

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Abstract

This research project was undertaken to address the knowledge gap that exists with regard to the impact of Australian livestock road transport practices on animal welfare. Five separate experiments were conducted to investigate the specific transport issues of: (i) loading practices (use of electric goad – cattle), (ii) transport duration (cattle 6 – 48 h and sheep 12 – 30 h) and (iii) pre-transport curfews – cattle and lambs (0, 12 and 24 h). The main conclusions were that loading and the initial phase of transport were the most stressful to livestock as indicated by the increase in blood cortisol concentration and body temperature. Whilst there was no additive effect of the use of electric goads on these measures at loading, it is recognised that electric goads will elicit a stress response when applied and therefore it is recommended their use be kept to a minimum in the interests of good animal welfare. The current maximum transport durations, which are based on the maximum period of water deprivation (48 h), within the welfare codes for cattle and sheep are acceptable on animal welfare grounds for the class of stock examined and the experimental conditions that prevailed.

Pre-transport curfews (period of enforced food and/or water deprivation) are applied to primarily reduce the gastrointestinal volume prior to transport, thus reducing the total amount of excreta in trucks and the level of faecal soiling on animals. Livestock transporters also advocate that curfews enable animals to cope better with the demands of transport. From the two studies conducted, pre-transport curfews neither enhanced nor compromised the capacity of animals to cope with transport. The period of curfew was merely additive in terms of the total time off feed and water. The need for pre-transport curfews should be predicated on consideration of key factors such as the nutritional background and condition of the cattle and sheep and the duration of the transport as well as the potential impacts on food safety. The research outputs have and will continue to have industry impact in the development of animal welfare standards pertaining to livestock transport and defence of these standards in the future.

Executive Summary

The livestock transport practices conducted in Australia are quite different to those in other countries. Moreover, there is a paucity of scientific evidence to allow conclusions to be drawn about the impact and relevance of these practices to animal welfare. Although Australian livestock may be well adapted to our environmental conditions, there are discrepancies between our practices and those of other countries and a lack of research or welfare assurance that demonstrates equivalence of outcomes. This research project was therefore undertaken with the goal to deliver scientifically defensible quantification of the animal welfare outcomes of Australian livestock transport practices. This in turn will enable the industry to defend against welfare-associated market access barriers, provide assurance to the general public and to markets and consumers and finally, identify any areas of potential concern.

The objectives (revised) of this research were:

1. Determine the contribution of handling, loading and initial transport processes on the stress responses of cattle to road transport;
2. Determine the animal welfare outcomes of yearling cattle transported under controlled conditions for 6, 12, 30 or 48 hours from farm to feedlot entry;
3. Determine the animal welfare outcomes of mature sheep (eg. live export trade) transported under controlled conditions for 12, 30 or 48 hours;
4. Investigate the interaction between pre-transport periods of food and water deprivation (curfew - 0, 12 and 24 h) and transport (12 and 24 h) in lambs and yearling cattle.
5. Develop recommendations on best practice yearling cattle and cast for age sheep transport management for optimal welfare and productivity.

The project comprised five separate experiments. The key transport issues investigated included (i) loading practices (use of electric goad – cattle), (ii) transport duration (cattle 6 – 48 h and sheep 12 – 30 h) and (iii) pre-transport curfews (0, 12 and 24 h).

The primary conclusions from this research were:

- The process of loading and the initial stages of the transport journey are the most stressful to both cattle and sheep. The application of an electric prodder to cattle during loading did not augment the overall stress response. The changes in blood cortisol concentration and body temperature indicate that loading was the most stressful aspect of transport. It is likely, that the specific effect of the electric prodder *per se* may have been overridden by the significant stress response that occurred during loading. This is not to say that the use of electric prodders is supported, rather it is recommended that their use should be kept to a minimum and only used as a last resort.
- Healthy mature cattle and sheep with no pre-transport feed or water curfew and transported in accordance with accepted good practice generally coped with transport durations up to 48 h. The current maximum duration of 36 h with the option to extend to 48 h (if the animals are not displaying obvious signs of fatigue, thirst or distress and if the extension allows the journey to be completed within 48 h) as stated within the Model Codes of Practice (Land Transportation of Cattle and Land Transportation of Sheep), is acceptable on

animal welfare grounds for the class of cattle and sheep examined in this project. However, it is important to emphasise that these results cannot be extrapolated across all transport events of this duration. The condition and physiological state of the livestock and the prevailing journey conditions are key variables that will influence the animals' response to transport. These variables must be considered in the transport planning phase.

- Subjecting healthy, grass-fed cattle or lambs to pre-transport periods of food and water deprivation (12 and 24 h compared with 0 h) prior to transport for 12 or 24 h did not adversely affect animal welfare. On the other hand in this study, pre-transport feed and water withdrawal did not enhance the capacity of the animals to cope with transport but simply added to the overall feed and water deprivation period and its associated effects. It is recommended that the need for pre-transport curfews should be predicated on consideration of key factors such as the nutritional background and condition of the cattle and sheep and the duration of the transport. In addition, they may also be required for slaughter stock to minimise the potential impacts of faecal soiling on food safety.

The new evidence from this project in relation to the impact of transport duration has enabled industry, government and regulatory agencies to make informed judgements about whether the current maximum durations within the cattle and sheep transport codes are appropriate on animal welfare grounds. Moreover, the planned publication of the research in internationally-recognised scientific journals will continue to place the industry in a good position to defend the welfare outcomes of the maximum transport durations for livestock permitted under Australian practice and law.

Contents

1 Background

The standards of husbandry and welfare practised during livestock production are becoming important factors influencing consumer perceptions in some markets (Kjaernes et al 2007). Clearly, the use of welfare-unfriendly livestock transport practices has the potential to downgrade product quality. For example, inappropriate stocking density during transport can cause increased carcass bruising in cattle (Eldridge and Winfield 1988). Furthermore, the use of practices that initiate market and public concerns that are unable to be adequately addressed may cause damage to the image and market access of Australian livestock products.

Prior to this research, the ability to demonstrate the welfare outcomes of livestock during land transport was limited. There was little scientific evidence that the welfare of cattle and sheep transported under Australian conditions was superior or equivalent to that of animals transported by other countries and international trading partners. Further, there are some marked differences in our conditions and practices compared with those of others. Long distances, heat and dust are regular features of livestock road transport in Australia, particularly for cattle in northern Australia. Previous Australian research conducted predominantly during the 1970's and 80's focussed on outcomes relating to carcass weight, muscle pH and bruising - important for productivity and quality, but not necessarily a complete validation of animal welfare. The European Union (EU) has transport intervals of 14 hours for sheep and cattle which contrast with the current Australian transport codes where livestock can be transported for up to 36-48 hours, with no provision for watering.

Although Australian livestock may be well adapted to our environmental conditions, the discrepancies between our practices and those of other countries may leave the Australian industry vulnerable, and reinforces the need for research to provide scientifically defensible quantification that demonstrates equivalence of outcomes. If Australian practices and regulations are going to be different of necessity from those of other trading blocs, then Australia needs objective evidence that the animal welfare outcomes are equivalent and acceptable.

In 2005, during the course of this research project, the World Society for the Protection of Animals (WSPA) announced their plans for an international campaign against long haul livestock transport. Therefore, it was prescient that this research was undertaken as it enabled the industry to have a solid foundation of scientific evidence about the welfare impacts of its road transport practices prior to the commencement of this campaign.

The goal of this project was to deliver scientifically defensible quantification of the animal welfare outcomes of Australian livestock transport practices. This in turn will assist the industry to:

1) defend against welfare-associated market access barriers,
2) provide assurance to the general public and to markets and consumers,
3) address any problems in practices that are revealed.

2 Project Objectives

The Objectives developed at the commencement of this project were as follows:

1. Determine the contribution of handling, loading and initial transport processes on the stress responses of cattle to road transport;

2. Determine the animal welfare outcomes of yearling cattle transported under controlled conditions for 6, 12, 30 or 48 hours from farm to feedlot entry;

3. Determine the animal welfare outcomes of mature sheep (eg. live export trade) transported under controlled conditions for 12, 30 or 48 hours;

4. Utilise a readily-applicable subset of welfare measures to determine the typical welfare outcomes of yearling cattle and cast for age sheep transported under typical commercial conditions; and

5. Develop recommendations on best practice yearling cattle and cast for age sheep transport management for optimal welfare and productivity.

During the course of the project, Objective 4 became supplanted by a need to examine the interaction of pre-transport feed and water withdrawal periods with transport duration. Pre-transport feed and water withdrawal is a common industry situation and practice, and thus contributes to the maximum time off water being examined as a critical issue by the project.

3 Methodology

The project comprised five separate experiments and the full reports for each are attached as Appendix A. The complete details of the methodology used in each experiment are described in these reports.

Experiment 1 - The effect of loading practices and 6-hour road transport on the physiological responses of yearling cattle.

Experiment 2 - Effect of road transport duration on indicators of cattle welfare.

Experiment 3 - Effect of road transport duration on indicators of sheep welfare.

Experiment 4 - The effect of pre-transport periods (0, 12 and 24 h) of food and water deprivation on the responses to 12 and 24 h of transport in yearling heifers.

Experiment 5 - The effect of pre-transport periods (0, 12 and 24 h) of food and water deprivation on the responses to 12 and 24 h of transport in Merino lambs.

4 Results and Discussion

The abstracts from each of the five experiment reports are presented to provide a synopsis of the experimental design, methods and key results. The detailed results and discussion for each experiment is provided in the reports contained within Appendix A.

Experiment 1 - The effect of loading practices and 6 hour road transport on the physiological responses of yearling cattle.

A controlled study using 16 yearling Angus steers was conducted to determine the physiological responses associated with different loading practices followed by 6 h of road transport and 17 h of post-transport recovery. The study was conducted in November 2004 on the New England Tablelands, NSW. The cattle were subjected to two treatments at loading: either 4 prods with an electric prodder or no prodding (control). The experiment was performed in four replicates, conducted on consecutive days, with four animals (n = 2 per treatment) utilised on each day. Blood samples were taken via jugular catheters before and during the 6h journey and during the 17-h recovery phase. These samples were analysed to determine haematology, osmolality and plasma cortisol, total protein, creatine kinase, blood urea nitrogen and the acute phase protein haptoglobin. The physiological measurements indicated that most stress occurred during loading and the initial stages of transport, but after this the cattle habituated and coped well with the 6 h of transport. After 17 h of recovery, nearly all the variables measured had returned to their pre-transport levels. The loading treatment had no effect on any of the measurements recorded. None of the post-transport values or rates of recovery in any of the physiological measurements indicate a welfare concern with 6 h of road transport for yearling cattle.

Experiment 2 - Effect of road transport duration on indicators of cattle welfare.

The aim of this experiment was to quantify the impact of transport duration on behavioural and physiological indicators of cattle welfare. The experiment was conducted in September/October 2005 in south-western Queensland. Two replicates of 4 transport duration treatments of 6, 12 and 30 and 48 h were examined. *Bos indicus* × *Bos taurus* heifers (n = 480; 383.5 ± 35.3 kg liveweight) were transported from one property to a feedlot using single trailer trucks (60 cattle/truck). On arrival, the cattle were allocated to pens with hay and water. Blood chemistry, haematology and liveweights were measured on 15 focal animals per vehicle pre-transport, and at 0, 24 and 72 h after arrival. Rectal temperature was logged during transport and for 72 h afterwards. Behaviour was recorded for 6 h post-arrival for the 12- and 48-h transport duration treatments. After 72 h, the cattle were regrouped and finished in the feedlot over 43 d. A significant ($P < 0.05$) interaction of transport duration × replicate × time was observed for the majority of the blood measurements and liveweight. The greatest difference between treatments was observed immediately on arrival, although this was not large for some measurements and generally within normal physiological values. There was a direct association between transport duration and liveweight loss, but by 72 h post-transport, the cattle had recovered more than 95% of their pre-transport liveweight. Compared to the 12-h transport group, the cattle transported 48 h spent significantly more time lying

and less time eating during the initial 3-h post-transport. The difference in lying time was less consistent and an opposite trend was observed for eating time during the second 3-h period. Most of the physiological measures had returned towards their pre-transport levels after 24 h of transport and further recovery was evident after 72 h particularly for the longer journey durations. The results of this study generally indicate that healthy cattle that have unrestricted access to food or water prior to transport can be transported with best practice for up to 48 h without major compromise to their welfare.

Experiment 3 - Effect of road transport duration on indicators of sheep welfare.

The aim was to determine the responses of healthy sheep to road transport under good conditions for 12, 30 or 48 h. The experiment was conducted in October/November 2006 in northern and north-western NSW. Merino ewes (n = 120; 46.9 ± 0.39 kg) were allocated to road transport treatments of 12, 30 or 48 h, with two replicates per treatment. Blood and urine samples and liveweights were taken pre-transport, and at 0, 24, 48 and 72 h after arrival. Lying time and vaginal temperature were measured using data loggers. Increasing transport duration resulted in increased haemoconcentration and indicators of catabolism, but that these effects did not exceed clinically normal ranges for any transport duration, and sheep generally recovered to pre-transport values within 72 h. Sheep transported for 30 and 48 h had lower liveweights on arrival than sheep transported for 12 h ($P < 0.001$). There were no differences between treatments in sheep liveweights at 24, 48 or 72 h after arrival. Sheep transported for 30 and 48 h had higher urine specific gravity and blood β-hydroxybutyrate concentrations on arrival than sheep transported for 12 h ($P < 0.001$). Sheep transported for 48 h had higher blood creatine kinase concentrations on arrival than sheep transported for 12 h ($P < 0.05$) although the levels were not high. Although white cell count and neutrophillymphocyte ratio increased with transport, there were no consistent effects of transport duration. There were no effects of transport duration on plasma cortisol concentrations. There were no differences in lying times during the first 6 h after arrival. Sheep transported for 30 or 48 h lay down for longer than sheep transported for 12 h between 7 and 15 h after arrival, but this effect was reversed later during the 24 h after arrival. Temperature profiles indicated a rise during loading and the initial stages of transport, followed by a decline to more basal temperatures. Most of the physiological measures had returned towards their pre-transport levels after 24 h of transport and further recovery was evident after 48 and 72 h particularly for the longer journey durations. These findings indicate that healthy adult sheep, transported under good conditions, can tolerate transport durations of up to 48 h, without undue compromise to their welfare.

Experiment 4 - The effect of pre-transport periods (0, 12 and 24 h) of food and water deprivation on the response to 12 and 24 h of transport in yearling heifers.

The aim of this experiment was to quantify the effect of pre-transport food and water deprivation (curfew) on the response to transport in yearling cattle. The experiment was conducted in July 2006 on the New England Tablelands, NSW. Eighty-four pasture fed yearling heifers were used for the experiment. A factorial design was used comprising three pre-transport food and water deprivation treatments of 0, 12 and 24 h and two transport duration treatments of 12 and 24 h, and these were replicated twice over a period of 4 weeks. At the conclusion of their transport treatments, the cattle were placed

in recovery pens with access to hay and water for 72 h. Detailed measurements of liveweight, blood chemistry, haemataology, urine concentration, body temperature, lying behaviour and water intake during recovery were recorded pre- and post-curfew, pre-transport and 0, 24, 48 and 72 h post-transport. The interaction between curfew treatment x transport treatment x time (sampling) was significant for many of the blood and urine measures, liveweight and behaviour. In general, the combined periods of curfew and transport were additive for their effects, particularly for liveweight loss and measures of hydration status (serum osmolality, urine specific gravity and osmolality) observed immediately post-transport. The incidence of lying behaviour during transport and the proportion of time lying during the initial period (0-3 h) of post-transport recovery were higher for cattle transported 24 h compared to 12 h. Furthermore, the incidence of lying behaviour in transit was higher in curfewed cattle, particularly those curfewed 24 h over the longer journey, than non-curfewed cattle. The results of this investigation indicate that subjecting healthy, grass-fed cattle to pre-transport periods of food and water deprivation (12 and 24 h compared with 0 h) prior to transport for 12 or 24 h did not enhance the capacity of the animals to cope with transport. The results also indicate that periods of food and water deprivation prior to transport are simply additive to transport-related periods of deprivation, and provide further evidence that healthy cattle under non-threatening environmental conditions can tolerate periods of up to 48 h of feed and water deprivation without undue compromise to their welfare.

Experiment 5 - The effect of pre-transport periods (0, 12 and 24 h) of food and water deprivation on the response to 12 and 24 h of transport in Merino lambs.

The aim of this experiment was to quantify the effect of pre-transport food and water deprivation (curfew) on the response to transport in Merino lambs. The experiment was conducted in May 2007 on the New England Tablelands, NSW. One hundred and eighty Merino lambs aged 6-7 months were used for the experiment. Of these, detailed measurements were made on 120 focal ewe lambs. A factorial design was used, comprising three pre-transport food and water deprivation treatments of 0, 12 and 24 h and two transport duration treatments of 12 and 24 h, and these were replicated twice over a period of 4 weeks. At the conclusion of their transport treatments, the lambs were placed in pastured paddocks with access to water for 72 h. Detailed measurements of liveweight, blood chemistry, haematology, body temperature, lying behaviour and water intake during recovery were recorded pre- and post-curfew, pre-transport and 0, 24, 48 and 72 h post-transport. Pre-transport curfews resulted in a significant reduction in liveweight and significant increase in serum cortisol, blood urea nitrogen and albumin concentrations. Total and differential white cell counts were also significantly affected. In the analysis of the pre- and post-transport data, several significant interactions were found for many of the measures. Notable here was the the interaction between curfew treatment x transport treatment x time (sampling) which was was significant for many of the blood measures (blood urea nitrogen, total protein, albumin, β-hydroxybutyrate, cortisol, white blood cell count, neutrophil and lymphocyte counts and neutrophil:lymphocyte ratio), liveweight and faecal score. The combined periods of curfew and transport were additive for their effects, particularly for liveweight loss observed immediately post-transport. This trend was less evident however for most of the other physiological measures, particularly those that indicate hydration status. Lambs that were curfewed (12 or 24 h) spent more time lying during journeys of 24 h

compared to non-curfewed animals. Curfew duration and transport (but not transport duration) resulted in a decrease in faecal scores reflecting a decrease in faecal moisture levels but did not affect microbiological characteristics of the faeces. The results of this investigation indicate that subjecting healthy, grass-fed lambs to pre-transport periods of food and water deprivation (12 and 24 h compared with 0 h) prior to transport for 12 or 24 h did not enhance the capacity of the animals to cope with transport.

General Summary

The main challenges for livestock during transport can include:

- Fear due to handling, loading and the conditions and novelty of transport
- Hydration, energy and fatigue challenges
- Thermal comfort and physical integrity

From the five experiments conducted there are some common outcomes:

1. The process of loading and initial stages of the journey were the most stressful based on the increase in blood cortisol concentration (hypathalamic-pituitary-adrenal (HPA) axis response) and body temperature (sympatho-adrenal (SA) response).
2. Cattle and sheep habituated to the journey conditions after a short period based on the decrease in these stress response measures.
3. Increasing the duration of transport and therefore water deprivation, resulted in haemoconcentration indicating some level of dehydration. However, the levels attained based on blood measures (eg. osmolality) were not a clinical concern and had returned to pre-transport levels within 72 hours after transport. The transport-mediated changes in blood measures of altered metabolic status were less consistent with respect to duration.
4. Most of the physiological measures had returned or were close to their pre-transport levels by 72 h post-transport.
5. Pre-transport curfews neither enhanced nor adversely affected the capacity of cattle and sheep to cope with transport under these experimental conditions. Instead they were additive with respect to the response to the total period of food and water deprivation.
6. Application of best practice transport principles facilitated the transport of cattle and sheep up to 48 h without undue compromise to their welfare.

Recovery after a 48-hour journey- assessment of suitable rest period.

A relevant question that has arisen in relation to this research project is the length of rest period that is suitable for animals to recover following a long (48-hour) journey, before they can be transported on a new journey. The current Codes (and the new Standards and Guidelines currently under development) allow animals to be transported for up to 48hrs and then following a rest period they could be transported on a second journey for up to 48 hrs. Therefore it is important that the recovery time is sufficient to allow animals to commence a second and possibly long journey.

The focus of the experiments conducted as part of this research was primarily on the effect on animals of journeys (incl. feed and water deprivation) of certain durations (e.g. 12, 30 & 48 h). Therefore the focus of the research was not to determine the ideal recovery time between one long journey and another (potentially) long journey. In this project, we did not transport animals, rest them and then transport them again and measure their responses to the second journey.

Nonetheless, it is our assessment across the experiments and many variables measured, that we can make a science-based interpretation of the data to help inform this issue. Variables were measured at intervals post-transport, enabling us to make assessments of the proportional recovery to pre-transport levels for each variable at each timepoint.

In considering recovery time following transport, we restricted the collation of the data to the two experiments (one in sheep and one in cattle) that focused solely on duration. This is because the two Curfew + Transport experiments comprised a 24-h curfew + 24-h transport for the treatment of greatest time off feed and water. Hence the post-transport recovery results from these studies are not directly comparable for this purpose with the transport duration data. The treatment schedule in the Curfew + Transport experiments meant that the animals were only transported for half of the total 48hr period off food and water., Therefore although responses such as hydration appeared to be similarly affected by curfew as for transport, the experience of being on a journey must inevitably affect other factors (such as muscular fatigue) much more than resting in a yard without feed or water.

Table 1 presents the proportional recovery (as percentages) of key variables following the 48-hour journeys in the cattle and sheep duration experiments. The variables were chosen to best reflect the major biological responses to long-distance transport: metabolic challenge, dehydration, and muscular fatigue. In interpreting the results, it is necessary to note that in the cattle study, blood samples were not collected at 48 h post-arrival. For both cattle and sheep, behaviour was only recorded in the hours immediately after arrival (for purposes of treatment comparison), and thus was unsuitable for estimating proportional recovery, because there was no baseline prior to transport to set at 100%.

Table 1. Percentage recoveries at fixed time points of key variables in response to 48 h of transport in cattle and sheep.

CATTLE			**Time post-arrival (h)**			
Variable (response)		**Pre-trans.**	**0**	**24**		**72**
Liveweight	(metabolic state)	100	90	97		96
Blood Urea Nitrogen	(metabolic state)	100	122	68		59
Betahydroxybutyrate	(metabolic state)	100	72	63		53
Plasma osmolality	(hydration)	100	103	101		98
Total plasma protein	(hydration)	100	105	97		97
Creatine kinase	(muscle fatigue)	100	237	166		84
SHEEP						
Variable (response)		**Pre-trans.**	**0**	**24**	**48**	**72**
Liveweight	(metabolic state)	100	88	92	92	91
Blood Urea Nitrogen	(metabolic state)	100	96	83	95	106
Betahydroxybutyrate	(metabolic state)	100	165	147	112	140
Plasma osmolality	(hydration)	100	102	100	101	103
Total plasma protein	(hydration)	100	109	100	100	101
Creatine kinase	(muscle fatigue)	100	139	102	82	76

The results indicate that some responses, such as hydration, recover extremely effectively by 24 h post transport. This is not surprising, as physiological studies show that animals given access to clean, palatable drinking water can recover their hydration status in a matter of hours following moderate periods of enforced water deprivation. The results indicate that responses indicative of metabolic status and muscular fatigue appear to take somewhat longer to recover. Sheep, perhaps because they are lighter animals, were more resistant to muscular fatigue, but took longer to recover metabolically. Cattle recovered more rapidly in metabolic terms, but had elevated creatine kinase levels for longer than sheep.

Overall, there was good recovery in the initial 24 h post-transport and further improvements thereafter. There was certainly variation in the recovery response, and from the data there was no consistent absolute time threshold where adequate recovery had been achieved. Having said that, taking into account the results and observations of the animals, it is our assessment that if animals have been transported close to the maximum duration (48 h), then 36 h of recovery would be more appropriate than 24 h before cattle or sheep can be transported further. The longer period of recovery will in our assessment ensure that the majority of animals have sufficiently recovered to cope

with a subsequent transport event, which under the Codes and the drafted Standards could be for another 48 h.

Recommendations on best practice cattle and sheep transport management for optimal welfare and productivity (Objective 5)

It is important for everyone involved in the preparation and transport of livestock to appreciate that transport can be stressful to animals. Best practice management of livestock transport results in good animal welfare and productivity.

The findings of this research project support the published recommendations for best practice livestock transport, as described by the MLA *Fit to Load* guide (available at www.mla.com.au) and the requirements of quality assurance schemes such as the TruckCare program of the Australian Livestock Transporters Association (www.alta.org.au).

The MLA-funded CSIRO research showed that it was possible to transport sheep and cattle for up to 48 hours without undue compromise to their welfare. It is important to note that there were a number of best practice components to the transport process that enabled this result to be achieved. These factors include:

There are 7 key elements of transport that need to be considered to minimise the impact on animals.

1. *Identification and selection of fit and healthy animals which can withstand the journey.*
The journey needs to be appropriate for the age, condition and physiological state of the animals. Young livestock must not be transported over long durations as they are more susceptible to the challenges associated with transport. Cattle or sheep in late pregnancy should not be transported on extended journeys. Other factors which need to be considered when determining the appropriate journey length for livestock include animal body condition, physiological state, hydration status, nutritional background, physical strength/robustness of the animals and wool cover particularly in relation to hot or cold conditions.

2. *Care during handling and loading.*
Livestock benefit from being loaded as quietly as possible, with the minimum use of dogs necessary to achieve animal flow. Use of low stress techniques to move stock based on an understanding of animal behaviours can make the loading process easier for stock and handlers. While it is not preferable to use dogs if they are used they should be kept under control at all times, and should be used in such a way to minimise potential injury to stock especially crowding which can lead to suffocation. Where electric prodders are necessary for cattle, these should be used sparingly. Good handling and loading facilities make the loading of livestock significantly easier for both handlers and animals, and promote low stress loading where use of prodders or dogs is unnecessary.

3. *Loading to appropriate stocking densities.*
Overcrowding of animals on transport vehicles has been shown to increase bruising, stress and injury during journeys. The recommended space allowances for cattle and sheep of different liveweights are shown in Table 1. Appropriate body size/class of stock

should be penned together to minimise risks of injury during transport. On very long journeys, or when weather conditions are predicted to be very hot, it is appropriate to allow animals slightly more space however giving animals too much space can reduce balance and stability of the pen group under some conditions leading to increased bruising. Practical experience needs to be used when determining appropriate stocking densities for a given journey.

4. Checking that the livestock are secured within the vehicle before the journey begins
These checks should also ensure that animals have not got their limbs, heads or horns trapped through the bars of the vehicle, and that loading has not resulted in any downed animals before the journey starts. Gates should be closed on all interior penning compartments within each deck to stabilise stock and minimise changes in stocking density due to animal movements during transport.

5. Appropriate driving techniques
Research has shown that livestock quickly adjust to a well managed journey, and learn to brace their stance for the movement of the vehicle. However, there is strong evidence that rough or unpredictable vehicle movements generally as a result of rapid braking, accelerating or cornering cause a greater incidence of animals slipping, losing their balance and falling.

6. Checking the livestock every few hours during a long journey
During a journey, it is possible for individual animals to go down and not immediately get up again, often due to the position of neighbouring animals. Regular checks (every 2 - 3 h) during a journey help to identify and encourage any such animals to regain their footing. Other problems can also be identified and rectified during regular checks.

7. Care during unloading and management on arrival
At the end of a journey, particularly a long trip, both sheep and cattle may be tired, and not as steady on their feet as during loading. Accordingly, animals need to be given appropriate time to move down ramps and off the vehicle. All animals should be provided with water and adequate space for rest after transport, particularly after long durations. The additional provision of feed will depend upon the intended use and condition of the livestock. Finally, animals must be allowed appropriate recovery periods with rest, feed and water before commencing another journey.

Table 1. Recommended space allowances during road transport for cattle and sheep (Draft Australian Standards and Guidelines for the Welfare of Animals, Land Transport of Livestock, Public Consultation Version)

Average liveweight (kg)	Minimum floor area (m^2/head)	
Cattle		Number of head per 12.2 m bottom deck
250	0.77	38
300	0.86	34
350	0.98	30
400	1.05	28
450	1.13	26
500	1.23	24
550	1.34	22
600	1.47	20
650	1.63	18
Sheep		
20	0.17	
30	0.19	
40	0.22	
50	0.25	
60	0.29	

5 Success in Achieving Objectives

The objectives as outlined in Section 2 have been achieved through the successful completion of the five experiments. The research focused on three specific transport issues; loading practices, transport duration and the interaction between pre-transport curfews and transport. The outcomes of these investigations and perhaps more importantly, the commonality of the results across cattle and sheep (refer Section 4) have enabled meaningful and robust conclusions to be drawn about these issues. This in turn, has been relevant and useful during the current process of redrafting of the Model Codes of Practice for Transport of Cattle and Sheep into Standards and Guidelines. It was recommended that future transport research should focus on how variables such as physiological state of the animal and on-board climatic conditions affect the response to transport duration.

In addition, the project facilitated the development and training of new scientific capability. Ms Sharon Pettiford, an MLA postgraduate, utilised experiments within this project to successfully complete her MSc studies. Ms Pettiford will graduate in 2008 and is now currently working on behalf of MLA in the Middle East. Furthermore, three journal manuscripts arising from the first 3 experiments have been submitted to Australian

Journal of Experimental Agriculture (Experiment 1) and are ready to be submitted to the Journal of Animal Science (Experiments 2 and 3, on MLA approval) for publication.

S.G. Pettiford, D.M. Ferguson, J.M. Lea, C. Lee, D.R. Paull, M.T. Reed, G.N. Hinch and A.D. Fisher (2008). The effect of loading practices and 6 hour road transport on the physiological responses of yearling cattle. (Australian Journal of Experimental Agriculture – accepted)

A.D. Fisher, D.O. Niemeyer, J.M. Lea, C. Lee, D.R. Paull, M.T. Reed, and D.M. Ferguson. (2008).
The effects of 12, 30 or 48 hours of road transport on the physiological and behavioral responses of sheep. (Journal of Animal Science – submitted).

D.M. Ferguson, S.G. Pettiford, D.D.O. Niemeyer, C. Lee, D.R. Paull, J.M. Lea, M.T. Reed and A.D. Fisher. (2008). Effect of duration of road transport on cattle behavior and physiology. (Journal of Animal Science – submitted).

6 Impact on Meat and Livestock Industry – now & in five years time

The goal of this project was to deliver scientifically defensible quantification of the animal welfare outcomes of Australian livestock transport practices. Having achieved this, it has already had an immediate industry impact. Under the auspices of the Australian Animal Welfare Strategy, all the current animal welfare model codes of practice will be redrafted into standards. These standards once enacted through the various state and territory governments will become law. The first welfare code currently going through this full process is the transport code that applies to all livestock. The new evidence from this project in relation to the impact of transport duration has enabled industry, government and regulatory agencies to make informed judgements about whether the current maximum durations within the cattle and sheep transport codes are appropriate on animal welfare grounds. The post-transport recovery results can also be applied to assess the validity of the recommended recovery times within the code for mature cattle and sheep before they can be subjected to further transport

In five years' time, the peer-reviewed, internationally-published information generated by this project will continue to place the industry in good position to defend the welfare outcomes of the maximum transport durations for livestock permitted under Australian practice and law.

7 Conclusions and Recommendations

The key conclusions and recommendations from this research are as follows:

The process of loading and the initial stages of the transport journey are the most stressful to both cattle and sheep. After this period, the physiological responses indicate that animals habituate to the journey conditions.

The application of an electric prodder to cattle during loading did not augment the overall stress response to loading and initial transport. This is not to say that the use of electric prodders is supported, rather it is recommended that their use should be kept to a minimum and only used as a last resort.

Healthy mature cattle and sheep with no pre-transport feed or water curfew and transported in accordance with accepted good practice generally coped with transport durations up to 48 h. Whilst those on the longer journeys (especially 48 h), were more tired and less hydrated, the physiological data indicates that they were not clinically compromised. It is concluded that the current maximum duration of 36 h with the option to extend to 48 h, which therefore means the maximum duration that can be undertaken in practice is 48 h, as stated within the Model Codes of Practice (Land Transportation of Cattle and Land Transportation of Sheep), is acceptable on animal welfare grounds for the class of cattle and sheep examined in this project.

Similarly, the findings during post-transport recovery support the codes recommendation of a 36 h rest period after transport for 48 h for both cattle and sheep.

Subjecting healthy, grass-fed cattle or lambs to pre-transport periods of food and water deprivation (12 and 24 h compared with 0 h) prior to transport for 12 or 24 h did not adversely affect animal welfare. On the other hand, the pre-transport feed and water withdrawal did not in itself enhance the capacity of the animals to cope with transport under these experimental conditions, it simply added to the overall feed and water deprivation period and its associated effects.

From a food safety perpsective, pre-transport curfews are beneficial through reductions in excreta volume and feacal mositure levels (Pethick et al 2006). However, these benefits can be partially offset by the observations that periods of food deprivation can in some cases lead to increased levels of enteric pathogens such as *E.coli* o157 and *Salmonella* (Pethick et al 2006). In the present research, the application of pre-transport curfews in lambs did not affect faecal microbiology suggesting that applying these feed and water deprivation and transport times will have little impact on the presence of *E. coli* and *Salmonella* in sheep faeces, and subsequently the risk of contamination of carcases.

The generic application of some period of pre-transport curfew, irrespective of the transport duration and nutritional background of the livestock, is questionable in terms of benefits. It is recommended that the need for pre-transport curfews should be predicated on consideration of key factors such as the nutritional background and condition of the cattle and sheep and the duration of the transport as well as requirements for condition of livestock destined for slaughter and possible impacts on level of animal soiling and associated food safety considerations.

Finally, it is recognised that the transport duration experiments were conducted using best practice and the animals did not experience extremes with regard to the on-board ambient conditions (THI range 50 – 80). This gives rise to the question, if the on-board conditions were more extreme (THI>80 or cold temperatures and wind chill were experienced), would this have influenced the animals' response? Indeed, this has been a frequently asked question at industry forums where this research has been presented. Issues around animal class, condition and preparation have also been raised. More specifically the transport of sheep over long journeys in winter, has been raised during the process of redrafting the transport codes into standards. There is no evidence to demonstrate how ambient conditions interact with the capacity of the animal to cope with extended journey durations. Therefore, it is recommended that further research and development be undertaken to specifically investigate how extreme ambient conditions (temperature, humidity and air movement) influence the behavioural and physiological responses in cattle and sheep during transport. It is proposed that this R&D could commence with the creation of a database of on-board ambient data in commercial livestock carriers operating during extreme temperature conditions. This database could then be used to model and research how these conditions would affect the animal's response.

8 Bibliography

Eldridge, G. A. & Winfield, C. G. 1988. The behaviour and bruising of cattle during transport at different space allowances. *Australian Journal of Experimental Agriculture* 28: 695-698.

Kjaernes, U, Roe, E and Bock, B (2007). Societal concerns on farm animal welfare. Assuring animal welfare: from societal concerns to implementation – 2nd Welfare Quality® Stakeholder Conference, Berlin Germany.

Pethick, D., Pointon, A., Kiermeier, A., Johns, M., Johns, J., Warner, R, Jacob, R., Ferguson, D., Paton, M. and Entwistle, K. (2006). LIVE122A Final Report- Investigating feed and water curfews for the transport of livestock within Australia – A literature review. (MLA, Sydney).

9 Appendices

9.1 Compiled Milestone Reports for the Five Experiments

Milestone 2

Report on the completion of the preliminary experiment examining the impact of pre-transport handling, loading, 6 hour transport and recovery

Abstract

The physiological responses associated with loading followed by 6 h of road transport and 17 h of post-transport recovery was examined in 16 Angus yearling steers. The cattle were subjected to two treatments on loading which included 4 prods with an electric prodder and no prodding (control). The physiological measures indicated that most stress occurred during loading and the initial stages of transport, but subsequent to this the cattle habituated and coped well with 6 h of transport. After 17 h of recovery, nearly all measurements had returned to their pre-transport levels. The loading treatment had no effect on any of the variables measured. None of the post-transport values or rates of recovery in any of the physiological measurements indicate a welfare concern with 6h of transport.

Project objectives

1. Determine the contribution of handling, loading and initial transport processes on the stress responses of cattle to road transport.
2. Determine the animal welfare outcomes of yearling cattle transported under controlled conditions for 6, 12, 30 or 48 hours from farm to feedlot entry.
3. Determine the animal welfare outcomes of mature sheep (eg. live export trade) transported under controlled conditions for 12, 30 or 48 hours.
4. Utilize a readily-applicable subset of welfare measures to determine the typical welfare outcomes of yearling cattle and cast for age sheep transported under a range of commercial conditions.
5. Develop recommendations on best practice yearling cattle and cast for age sheep transport management for optimal welfare and productivity.

Success in achieving milestone

THE EFFECT OF DIFFERENT LOADING PRACTICES AND 6HR ROAD TRANSPORT ON PHYSIOLOGICAL RESPONSES IN CATTLE

Sharon Pettiford, Drewe Ferguson, Andrew Fisher, Caroline Lee, David Paull, Jim Lea and Matt Reed

1. Introduction

The standards of husbandry and welfare practiced during livestock production are becoming important factors influencing consumer perceptions in many markets. Clearly, the use of welfare-unfriendly livestock transport practices has the potential to downgrade product quality. Furthermore, the use of practices that initiate market and public concerns that are unable to be adequately addressed may cause damage to the image and market access of Australian livestock products.

Unfortunately, the ability of Australian livestock industries to demonstrate the welfare status of livestock during land transport remains vulnerable on several grounds. There is little scientific evidence that the welfare of cattle and sheep trucked under Australian conditions is superior or equivalent to that of animals transported by our competitors and international trading partners. Further, there are some marked differences in our conditions and practices compared with those of others. If Australian practices and

regulations are going to be different of necessity from those of other trading blocs, then we may need objective evidence that the animal welfare outcomes are equivalent and acceptable. Consequently this project was developed to develop scientifically defensible quantification of the animal welfare outcomes of Australian livestock transport practices. The research reported here represents the first in a series of investigations examining the animal welfare outcomes of land transport practices for cattle and sheep.

The specific aim of this study was to quantify the stress responses in cattle during and subsequent to 6 hours of road transport. Particular emphasis was placed on examining the physiological responses during the initial transport processes where the hypothesis that handling method influenced the stress response during the initial stages of transport was tested.

2. Materials and Methods

The experiment was approved by the CSIRO Livestock Industries FD McMaster Laboratory Animal Ethics Committee (AEC No. 04/41).

2.1 Cattle

Twenty Angus yearling steers were purchased from a local property and backgrounded at the CSIRO Livestock Industries (CLI) FD McMaster field station for 3 weeks prior to the commencement of the transport experiment. The mean $\pm$ se liveweight of the steers was 299 $\pm$ 2.6 kg.

2.2 Modifications to the truck

The CSIRO Armidale stock truck was used for the experiment. The stock crate was modified such that four individual pens were built within the crate (see Figure 1). All four pens were identical in their design and each allowed the animals to stand in their own compartment during the journey. 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 two temperature and humidity loggers (Tiny Tag plus TGP1500 loggers - temperature range -30 – 50^{o}C and relative humidity 0-100%). One logger was fitted within the crate and the other was positioned externally under the truck crate.

Figure 1 Modifications to stock crate and cattle position during transport

2.3 Experimental design

Sixteen animals were randomly allocated to one of two loading treatments and there were four journey replicates. The cattle in one group (Elec. Prodder) received 4 prods using an electric prodder in the race prior to the loading ramp, whilst the other group (Control) received no prodding and were quietly loaded onto the truck. The experiment was conducted over 4 days, where four animals/day were transported for 6 hours and a total distance of 273 km. The truck journey consisted of a 25 min circuit which facilitated collection of blood samples and behavioural observations approximately 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 pastured paddock. On the following morning (08:00), the cattle were returned to the yards for removal of the temperature loggers and final blood sampling.

2.4 Blood sampling and biochemical measurements

The experimental animals had an intravenous jugular catheter inserted the day before transport. Throughout the duration of the experiment, each animal was blood sampled via the catheter before loading (-1hr), loading (0hr), during transport (0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5 & 6 h), after transport (7, 9, 12 and 23 h). Two blood samples were collected at each of these time points in serum separation monovettes and EDTA monovettes. Prior to centrifuging, whole blood was analysed for haematology variables (white blood cells (WBC), neutrophils, lymphocytes, monocytes, eosonophils, basophils, red blood cells (RBC), haemaglobin (HGB), haematocrit (HCT)) using a Cell-Dyn Haematology Analyser 3500R (Abbott Diagnostics, CA, USA).

After centrifuging, the serum and plasma were harvested and frozen (-20^{o}C) until they were required for analysis. The 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 only assessed at 5 time points (-1, 1, 3, 6 and 23 h).

The serum concentration of cortisol was determined using a Spectria Cortisol RIA (Orion Dianostica, Espoo, FIN). A DADE Behring ACA ® clinical autoanalyzer (Walton Manor, UK) was used to analyse the blood urea nitrogen, creatine kinase and total protein plasma concentrations. Osmolality was measured using a vapour pressure osmometer (Wescor, 5500). The acute phase protein haptoglobin was determined in plasma by a variation of the method of Jones and Mould (1984). The assay was modified to account for the effect of free haemoglobin due to haemolysis (Slocombe and Colditz 2005).

2.5 Body temperature

On the day prior to transport, cattle were fitted with temperature loggers and harnesses to record body temperature. Vemco Minilog M108-TXC temperature loggers were fitted into probes that were placed in the rectum and secured in place via elastic cord attached to a girth harness (see Figure 2). The instrumentation allowed normal defaecation by the animals. Body temperature was recorded every 3 min until the harnesses and probes were removed at 23 h post-transport.

Figure 2 Cattle fitted with harnesses and rectal temperature loggers.

2.6 Statistical Analysis

Several blood parameters were normalized prior to the analysis via log (neutrophils %, lymphocytes %, monocytes %, eosinophils %, haemoglobin concentration, haematocrit %, creatine kinase and blood urea nitrogen concentrations) or square root (Basophil %) transformations. The data were analysed using the mixed model procedure in SAS (SAS

Institute Inc., Cary, NC, USA.). The initial model contained the fixed effects of loading treatment, journey replicate (Day), blood sampling time (Time) and their interactions plus a random term for animal. Non-significant interactions were sequentially removed to reveal the final model. For body temperature, the measurement that coincided with each of the 18 blood sampling times was extracted from the temperature profiles and analysed.

3. Results

The on-board temperature conditions varied somewhat between the days of the study (Figure 3). The cooler temperatures experienced on Friday coincided with rain and overcast conditions.

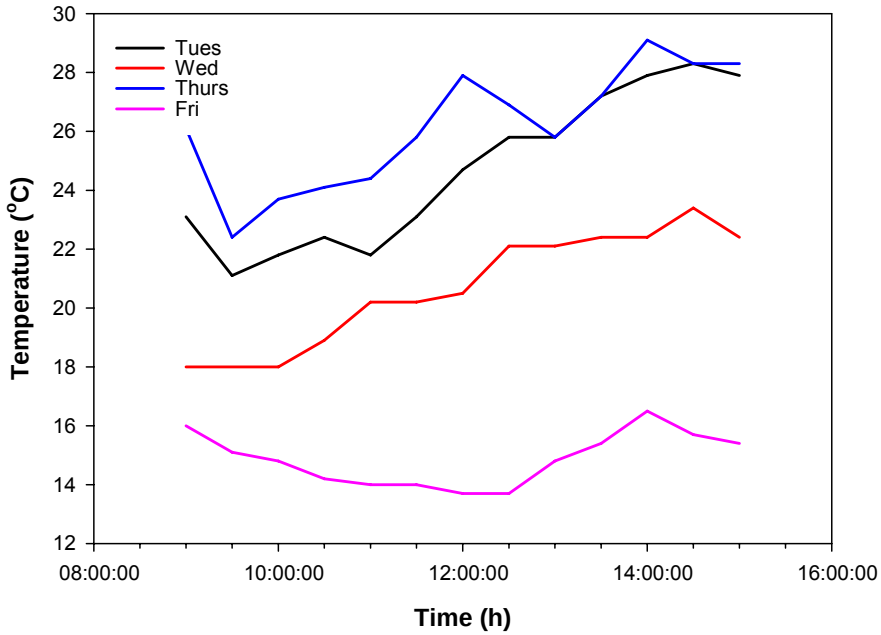


Figure 3 Ambient temperature profile (in crate temperature logger) during the 6 h of transport over the four days.

3.1 Effect of loading treatment

The effect of loading treatment had no significant effect on rectal temperature and the majority of the blood measurements (Tables 1 and 2). The notable exception here was WBC (Figure 4) where the effect of the loading treatments was influenced by day of transport ($P<0.05$). Although there was a contrasting trend between the treatments on Tuesday and Wednesday compared to Thursday and Friday, the interaction was largely the result of differences between the individual treatments between days. Also a significant three-way interaction ($P<0.01$) between loading treatment × day × time was observed for plasma total protein (Figure 5).

Table 1 Effect of loading treatment, day, sampling time and their interactions on the body temperature and the plasma concentrations of cortisol (nmol/l), total protein (g/dl), creatine kinase (U/l) and blood urea nitrogen (BUN mg/dl).

	Cortisol	Tot. Protein	CK*	BUN*	Temp.
Main Effects					
Load. Treat. (LT)					
Control	39.11	6.79	426.75	13.67	39.23
Elec. Prodder	43.14	6.94	453.87	13.58	39.23
SED	5.60	0.1	0.15	0.06	0.12
Significance	*ns*	*ns*	*ns*	*ns*	*ns*
Day					
Tuesday	36.07	6.93	439.88	15.13	39.41
Wednesday	43.03	6.92	529.38	13.23	39.15
Thursday	45.22	6.88	409.12	13.49	39.22
Friday	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					
-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	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.001*
Interactions					
LT × Day	*ns*	*ns*	*ns*	*ns*	*ns*
Day × Time	*P<0.05*	*P<0.01*	*ns*	*ns*	*P<0.05*
LT × Time	*ns*	*ns*	*ns*	*ns*	*ns*
LT × Day × Time	*ns*	*P<0.01*	*ns*	*ns*	*ns*

*Backtransformed means shown

Table 2 Effect of loading treatment, day, sampling time and their interactions on haematology parameters, osmolality and haptoglobin concentration

	WBC	Neut.*	Lym.*	Mono.*	Eosin.	Baso.*	RBC	HGB*	HCT*	Osmol.	Hapt.*
Main Effects											
Load. Treat. (LT)											
Control	9.34	4.21	3.57	0.77	0.10	0.09	7.27	11.13	29.49	279.39	3.46
Elec. Prodder	9.72	4.18	3.85	0.77	0.07	0.11	7.69	11.52	30.76	277.99	3.68
SED	0.52	0.12	0.13	0.14	0.04	0.02	0.04	0.02	0.02	0.05	0.21
Significance	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
Day											
Tuesday	8.50	4.27	3.29	0.70	0.03	0.07	7.22	11.29	30.37	282.81	3.18
Wednesday	9.27	3.65	3.90	0.80	0.08	0.11	7.82	11.55	30.63	269.81	4.12
Thursday	9.15	3.58	3.82	0.77	0.08	0.10	7.19	10.95	29.32	269.65	3.00
Friday	11.21	5.57	3.88	0.82	3.23	0.13	7.69	11.50	30.16	292.79	4.12
SED	0.74	0.17	0.18	0.20	0.05	0.03	0.06	0.03	0.03	0.06	0.30
Significance	*P<0.05*	*ns*	*ns*	*ns*	*ns*	*P<0.05*	*ns*	*ns*	*ns*	*P<0.005*	*ns*
Time											
-1	7.75	2.24	4.23	0.65	0.09	0.08	7.91	11.98	31.93	275.99	2.55
1	9.25	4.27	3.75	0.68	0.07	0.13	7.51	11.38	30.17	279.39	2.09
3	10.49	6.27	3.29	0.76	0.05	0.13	7.19	10.88	28.76	279.52	2.50
6	11.04	6.48	3.25	0.88	0.007	0.10	7.29	11.03	29.24	280.36	3.59
23	8.84	3.36	4.15	0.92	0.21	0.07	7.48	11.37	30.58	278.19	12.07
SED	0.39	0.11	0.07	0.09	0.02	0.01	0.01	0.01	0.01	0.05	0.16
Significance	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.001*	*ns*	*P<0.001*
Interactions											
LT × Day	*P<0.05*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
Day × Time	*P<0.05*	*ns*	*ns*	*ns*	*ns*	*ns*	*P<0.05*	*P<0.01*	*P<0.05*	*P<0.001*	*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*

*Backtransformed means shown
WBC- white blood cell count (x 10^9/l), Neut.- neutrophils %, Lym.- lymphoctes %, Mono.-moncytes %, Eosin.-eosinophils %, Baso.-basophils %, RBC-red blood cell count (x 10^{12}/l), HGB-haemoglobin (g/dl), HCT- haematocrit %, Osmol.-osmolality (mOsmol.) and Hapt.-haptoglobin (mg/dl)

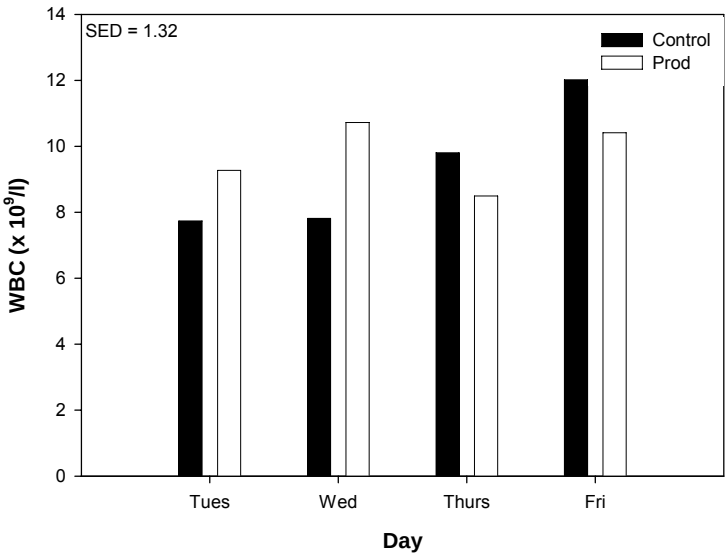


Figure 4 Differences between days on the effect of loading treatment on white blood cell count (WBC) in response to 6 h transport and during 17 h recovery

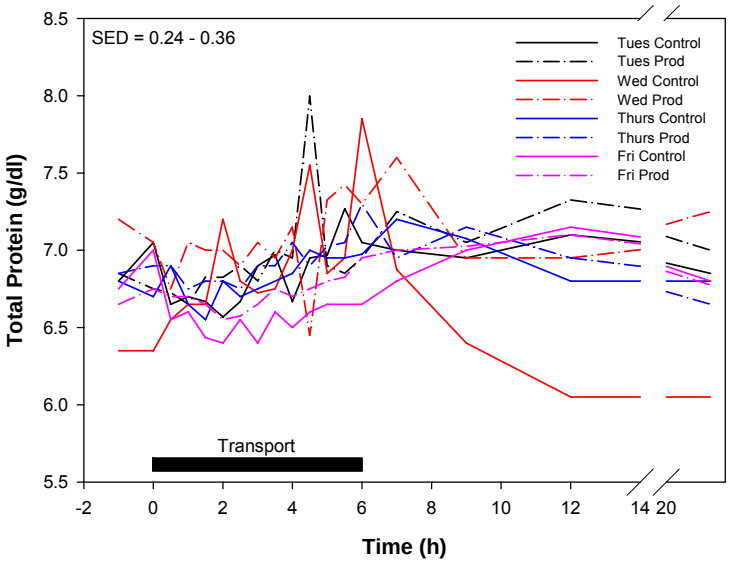


Figure 5 Effect of the interaction between loading treatment × day × sampling time on plasma total protein concentration.

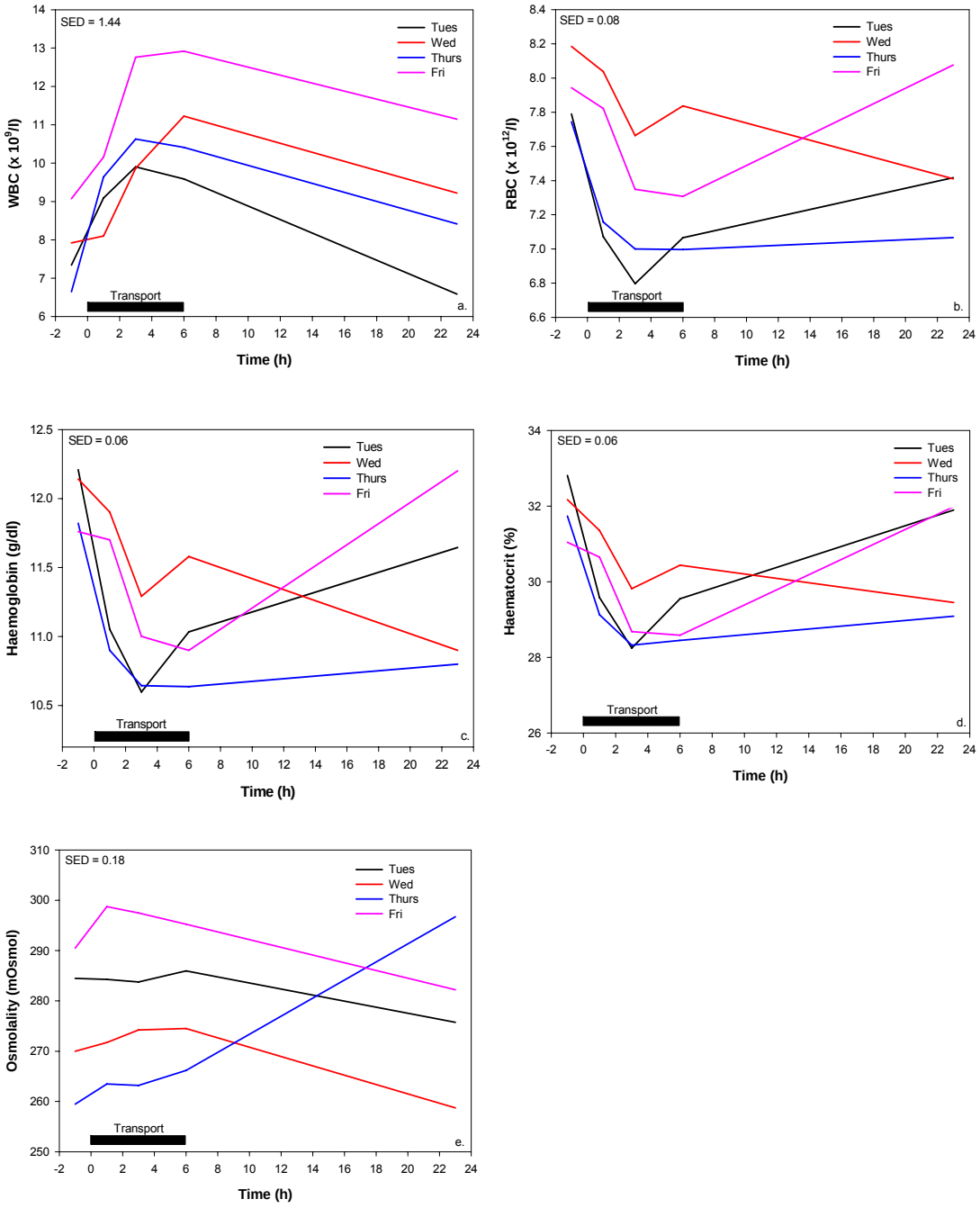


Figure 6abcde Differences between days on the changes in white blood cell count (WBC), red blood cell count (RBC), haemoglobin concentration (HGB), haematocrit (HCT) and osmolality in response to 6 h transport and during 17 h recovery.

3.2 Effect of day of transport

A significant day × time interaction was observed for several measures (WBC, RBC, haemoglobin, haematocrit, osmolality, cortisol and temperature) indicating that the changes over time in response to transport and during recovery differed between days (Figure 6abcde and Figure 7ab). There was no consistent trend between days across the various measurements. In general, the effect of transport day had no significant effect on the physiological measures with the exception of Basophils % ($P<0.05$).

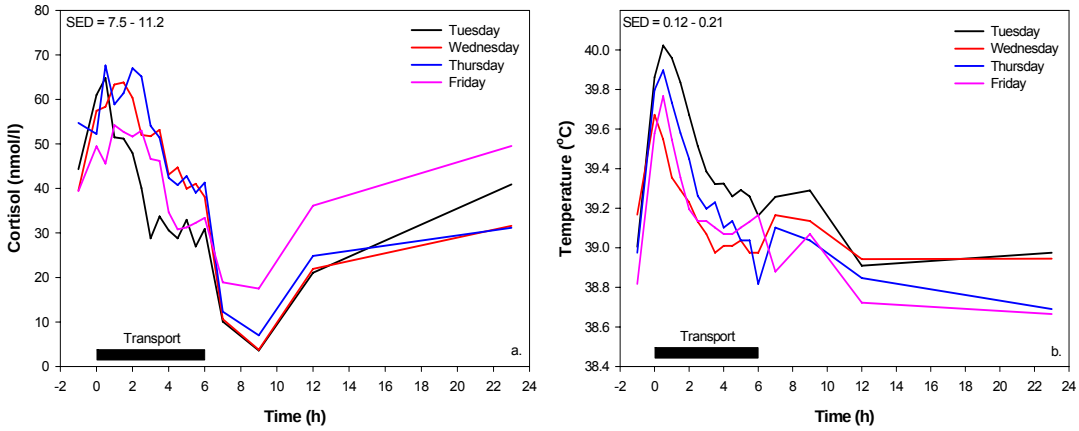


Figure 7ab Effects of time and differences between days on the changes to serum cortisol concentration and body temperature in response to 6 h transport and during 17 h recovery.

3.3 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 minimal effect on the hydration status of the animals. The change in these variables after transport ranged from 1-5% (Table 3). Plasma blood urea nitrogen (BUN) significantly declined during the 6 h of transport (Figure 8). The stress indicators of plasma cortisol and body temperature showed similar response patterns particularly during the loading and initial stages of transport (Figure 7ab). 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 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 muscle activity and damage (Figure 8).

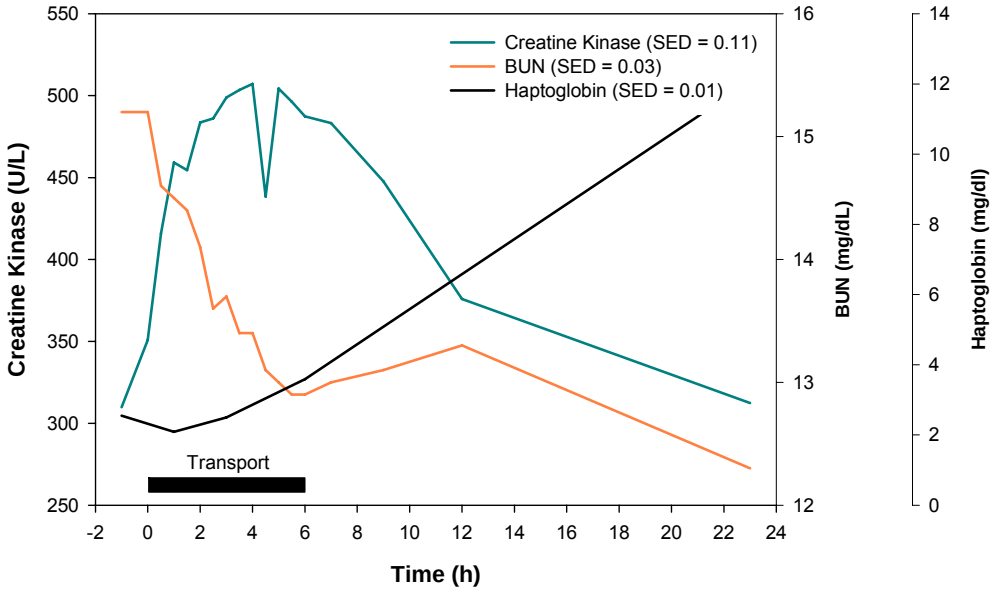


Figure 8 Changes in creatine kinase, blood urea nitrogen (BUN) and haptoglobin concentrations in response to 6 h transport and 17 h recovery.

Table 3 Percentage change in selected physiological measurements after 6 h transport*

Measurement	Change
WBC (x 10^9/L)	42.5 % increase
RBC (x 10^{12}/L)	6.5 % increase
Haemoglobin (g/dL)	1.9 % increase
Haematocrit %	0.7 % decrease
Osmolality (mOsmol.)	3.5 % 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)	15.1 % decrease
Temperature (°C).	-

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

3.4 Post-transport recovery

After 23 h after the commencement of transport, most measurements had returned to their pre-transport basal 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.

4. Discussion

The increase in blood cortisol and body temperature during loading and the initial stages of transport indicate that these aspects were stressful to the cattle. However, the attenuation in the responses during the journey also indicates that the cattle habituated and coped with 6 h of transport. Moreover, it was interesting to note that unloading did not elicit any major change to either cortisol or temperature. These observations are generally consistent with those from other cattle (Eldridge et al 1988, Warriss et al 1995) and sheep (Broom et al 1996) transport studies. In summary, loading and the initial transport stages are the most stressful and that over short to moderate journey lengths, the animals generally adapt.

The stress associated with transport can also account for the increase in total white blood cell count and neutrophil %. Burrow et al (1998) reported similar findings although their differential cell counts were much higher when cattle were trucked over a much greater distance and handled significantly more than in the present study.

The rise in creatine kinase over the 6h indicates that the exertions during loading and the maintenance of the balance during the journey were physically demanding. 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 Wariss 2000). Wariss et al (1995) showed that plasma levels of creatine kinase were positively associated with journey length (5 – 15 h) and reported a 270% increase in creatine kinase levels over 5 h of transport. This is markedly higher than that observed in the present study (57%). It is also worth mentioning that whilst creatine kinase is a useful marker of muscle activity it is by no means a definitive indicator of muscle fatigue. Changes in muscle glycogen concentration would be more informative in this context.

The small changes in serum osmolality, total protein, haemoglobin or haematocrit % indicate that 6 h of transport has had minimal impact on the hydration status of the cattle. Similarly, there was no evidence of any transport stress and/or fasting induced protein catabolism as reflected by the changes in blood urea nitrogen. The significant decrease in blood urea nitrogen contrasts with the 6.5% increase observed by Warriss et al (1995) after 5 h transport.

The use of an electric prodder on loading did not produce any significant effects on the variables measured. This is not to say that prodding is desirable, but its effects are probably more transient in altering animal behaviour, possibly making them more agitated and difficult to handle. It is also likely, that the specific effect of prodding *per se* may have been overridden by the significant stress response that occurred during loading.

5. Conclusions

The results of this study lead us to conclude that 4 prods with an electric prodder during loading did not augment the overall stress response to loading and initial transport. This is not to say that the use of electric prodders is supported, rather their use should be kept to a minimum.

Loading and the initial stages of transport were the most stressful but cattle generally adapted quite well to 6 h of transport.

Overall, none of the post-transport values or rates of recovery in any of the physiological measurements indicate a welfare concern with 6h of transport.

6. References

Broom, D.M., Goode, J.A., Hall, S.J.G., Lloyd, D.M and Parrott, R.F. (1996). Hormonal and physiological effects of a 15 hour road journey in sheep: comparison with the responses to loading, handling and penning in the absence of transport. *Br. Vet. J.* **152**:593-605.

Burrow, H.M., Colditz, I.G and Oddy, V.H. (2005). Relationships between temperament and haematological values and the effects of an electrolyte replacement treatment on weight loss during, and recovery following, long distance transport of *Bos indicus* and Zebu derived weaner cattle. *Aust. J. Agric. Res*. (submitted).

Eldridge, G.A., Winfield, C.G. and Cahill, D.J. (1988). Responses of cattle to different space allowances, pen sizes and road conditions during transport. *Aust. J. Exper. Agric.* **28**:155-159.

Jones, G.E. and Mould, D.L. (1984). Adaptation of the guaiacol (peroxidase) test for haptoglobins in a microtitration plate system. *Res. Vet. Sci*. **37**:87-92.

Knowles, T.G. and Wariss, P.D. (2000). Stress physiology of animals during transport. In: "Livestock Handling and Transport" 2nd Edition. Ed T.Grandin, (CAB International, Oxon UK).

Slocombe, L.L. and Colditz, I.G. (2005). Evaluating the stress of production in cattle using haptoglobin. 5th Annual International Colloquium on Animal Acute Phase Proteins, Dublin, Ireland.

Warriss, P.D., Brown, S.N., Knowles, T.G., Kestiin, S.C., Edwards, J.E., Dolan, S.K. and Phillips, A.J. (1995). Effects on cattle of transport by road for up to 15 hours. *The Vet. Rec.* **126**:319-323.

Overall progress of the project

Good progress has been made on the project to date. With regard to the stage 1 cattle research, the project team met with senior managers from NAPCO (Geoff Kingston, General Manager – Growing and Marketing and Geoff Cornford, Feedlot Manager) to discuss possible collaboration. The response was very favourable and further discussions are taking place. Contingent on the outcomes of these discussions it is anticipated that the research will commence on schedule in June 2005.

Recommendations

Appendices

Milestone 4

Report on the completion of the stage 1 cattle study examining the effect of transport duration on animal welfare indicators. Recommendations on the most informative welfare indicators for further validation in stage 2.

Abstract

The aim of this experiment was to quantify the impact of transport duration on behavioral and physiological indicators of cattle welfare. Four hundred and eighty NAPCO heifers (mean liveweight 383.5 ± 35.3 kg) were used for the experiment. The design comprised 4 transport duration treatments of 6, 12 and 30 and 48 h and these were replicated over 2 weeks. The cattle were transported from their backgrounding property to the NAPCO feedlot using single trailer commercial trucks. The departure times were staggered to allow the trucks to arrive at the feedlot on the same day. On arrival, the cattle were allocated to pens with access to hay and water. Detailed measurements including liveweight, blood chemistry and haematology were collected on 15 focal animals on each vehicle pre-transport, immediately on arrival and 24 and 72 h after arrival. Rectal temperature was logged over the entire transport and 72 h post-transport phases. After the 72 h recovery phase, the cattle were regrouped and finished in the feedlot over 43 days prior to slaughter. Variation was observed between the transport treatments and the weekly replicates for some of the pre-transport blood chemistry parameters. On arrival, a significant ($P<0.05$) 3-way interaction between transport duration x week x sampling time was observed for the majority of the blood chemistry and haematology measurements and also for liveweight. The largest variation between the transport duration treatments was observed immediately on arrival but in many cases these were not large and often still within the normal physiological ranges for many of the blood parameters. There was positive trend between transport duration and liveweight loss however, the differences between the treatments were not always significant. The cattle recovered the majority (95 – 98%) of the weight lost through transport by 72 h after transport. Cattle transported 48 h spent significantly more time lying during the initial 3 h post-transport phase but the trend was less consistent during the second 3 h period as the results differed between weeks. Transport duration had no effect on feedlot average daily gain. The results of this study generally indicate that healthy cattle that have not had restricted access to food or water prior to transport can tolerate transport up to 48 h without any major compromise to their welfare.

Success in achieving milestone

THE EFFECT OF TRANSPORT DURATION ON INDICATORS OF CATTLE WELFARE

Sharon Pettiford, Drewe Ferguson, Andrew Fisher, Caroline Lee, David Paull, Jim Lea and Matt Reed

1. Introduction

The standards of husbandry and welfare practiced during livestock production are becoming important factors influencing consumer perceptions in many markets. Clearly,

the use of welfare-unfriendly livestock transport practices has the potential to downgrade product quality. Furthermore, the use of practices that initiate market and public concerns that are unable to be adequately addressed may cause damage to the image and market access of Australian livestock products.

Unfortunately, the ability of Australian livestock industries to demonstrate the welfare status of livestock during land transport remains vulnerable on several grounds. There is little scientific evidence that the welfare of cattle and sheep trucked under Australian conditions is superior or equivalent to that of animals transported by our competitors and international trading partners. Further, there are some marked differences in our conditions and practices compared with those of others. If Australian practices and regulations are going to be different of necessity from those of other trading blocs, then we may need objective evidence that the animal welfare outcomes are equivalent and acceptable. Consequently this project was designed to develop scientifically defensible quantification of the animal welfare outcomes of Australian livestock transport practices. The research reported here represents the first in a series of investigations examining the animal welfare outcomes of land transport practices for cattle and sheep.

In Australia, livestock can be transported over considerable distances and durations. Under the Australian Model Code of Practice for the Land Transportation of Cattle, the maximum allowable duration is primarily determined by the maximum time that cattle can be deprived of water. For mature dry cattle, the maximum duration is 36 h. However, this can be extended to 48 h if the animals are not displaying obvious signs of fatigue, thirst or distress and if the extension allows the journey to be completed within 48 h. The Australian evidence supporting the establishment of these maximum periods is very limited and indirect, in that it was probably derived from cattle dehydration data. Given the paucity of direct evidence, this investigation was undertaken to specifically examine the impact of transport duration on cattle welfare indicators.

2. Materials and Methods

The experiment was approved by the CSIRO Livestock Industries Rockhampton Animal Experimentation Ethics Committee (Approval No. RH 210/05). The experiment was conducted in collaboration with the Northern Australia Pastoral Company (NAPCO).

2.1 Cattle

Four hundred and eighty NAPCO composite heifers were sourced for the experiment. The cattle were sourced from 2 NAPCO properties, *Marion Downs* (n=240) and *Coorabulka* (n=240) which are located in the Channel Country in south-western Queensland. They were transported to the NAPCO backgrounding property *Lanreef*, near Roma. This journey comprised a 6-7h trip to Winton where the cattle were unloaded and spelled in the yards for 15-20h followed by a further 20h transport to *Lanreef*. The cattle arrived on 18th July 2005 (from *Marion Downs*) and 15th August 2005 (from *Coorabulka*) and the two groups were mixed on the 22nd August 2005. After a day together, the cattle were drafted into 2 groups of 240 animals, balanced for property of origin. The cattle were then maintained in their groups on improved pasture up until the commencement of the experiment (19th September 2005). The average

liveweight of each group prior to the commencement of the transport treatments was 388.7 ± 35.8 and 378.2 ± 34.2 kg.

2.2 Experimental design and procedures

The design comprised 4 transport duration treatments of 6, 12 and 30 and 48 h and these were replicated over 2 weeks. Specific transport routes between *Lanreef* and the NAPCO feedlot *Wainui* were identified in consultation with the commercial transport company (Frasers Transport, Warwick) to achieve the desired transport duration. Four single trailer trucks with Byrne stockcrates (capacity 60 head) were used each week. The departure times for each truck/treatment were staggered so that the 4 trucks arrived at *Wainui* on the same day.

The experimental schedule for each week is shown in Appendix 1. On each week, one day prior to the commencement of the transport treatments, one group of 240 head was yarded and the cattle were weighed and measured for flight time. The cattle were then randomly allocated, stratified for liveweight and flight time, to 1 of the 4 transport duration treatments (60 head/treatment). Within each transport treatment sub-group, 15 focal animals were randomly selected and identified. Each sub-group was returned to separate holding paddocks with access to water and improved pasture. Each sub-group was returned to the yards 1.5 h prior to the commencement of their transport treatment. The cattle were weighed and the focal animals were fitted with rectal temperature loggers and blood sampled. The sub-group including the focal animals were then loaded onto the truck. The focal animals were randomly allocated to the front and rear compartments on both decks.

Each truck was fitted with a GPS logger and a temperature and humidity logger (Tiny Tag plus TGP1500 loggers - temperature range -30 to +50^{o}C and relative humidity 0 to 100%). The latter was located on the roof of the front compartment in the lower deck.

Upon arrival at the feedlot, the cattle were unloaded and immediately weighed. Blood samples were collected on the focal animals and for some, the rectal temperature loggers had to be refitted or adjusted. The focal animals from each transport duration treatment were then placed in a small holding yard with access to water and hay. The remainder of each treatment sub-group was held in a larger holding yard with water and hay provided. The focal animals were weighed and blood sampled again 24 and 72 h post transport after which, the temperature loggers were removed and the cattle were returned to the larger group.

The weekly group (n=240) was then inducted into the feedlot 2 days after transport. Each group was maintained in a single feedlot pen and fed a commercial feedlot ration for 43 days before the group was transported to the abattoir for slaughter.

2.3 Blood sampling and biochemical measurements

Each of the focal animals was blood sampled on 4 occasions (pre-transport, arrival and 24 and 72 h post-transport). At each sampling, 6ml and 10 ml (EDTA vacutainers) samples were collected. An aliquot of whole blood was analysed for haematology variables (haemoglobin (HGB), red cell counts (RCC), haematocrit (HCT), mean

corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelet count (PC), white blood cells count (WBC) and differential cell counts of neutrophils (NEU), lymphocytes (LYM), monocytes (MON), eosonophils (EOS), basophils (BAS)) using a Cell-Dyn Haematology Analyser 3500R (Abbott Diagnostics, CA, USA).

An aliquot of blood was centrifuged and the resultant plasma was harvested and frozen (-20^{o}C) for subsequent analysis of blood chemistry. The buffy coat containing the white blood cells was also sampled and RNA was extracted to examine changes in gene expression.

The concentrations of cortisol, blood urea nitrogen, total protein, albumin, creatine kinase, β-hydroxy butyrate and haptoglobin were determined on the plasma samples. Cortisol was determined using a Spectria Cortisol RIA (Orion Dianostica, Espoo, FIN). A DADE Behring ACA® clinical autoanalyzer (Walton Manor, UK) was used to analyse the blood urea nitrogen, creatine kinase and total protein plasma concentrations. Osmolality was also measured using a vapour pressure osmometer (Wescor, 5500). The acute phase protein haptoglobin was determined in plasma by a variation of the method of Jones and Mould (1984). The assay was modified to account for the effect of free haemoglobin due to haemolysis (Slocombe and Colditz, 2005).

2.4 Body temperature

On the day of transport, focal cattle were fitted with temperature loggers and harnesses to record body temperature. Temperature loggers (Thermochron iButton, Maxim Integrated Products, USA) were secured to probes that were placed in the rectum and secured in place via elastic cord attached to a girth harness. The instrumentation allowed normal defaecation by the animals. Body temperature was recorded every 2-3 min until the harnesses and probes were removed at 72 h post-transport.

2.5 Post-transport behavioural observations

Behaviour was monitored over the immediate 6-7h post-transport period for 2 of the transport duration treatments (12 and 48 h). It was not feasible to collect behavioural data on all 4 treatment groups and the contrast between these 2 groups was considered the most useful. Video cameras were mounted above the pens and these continuously recorded animal behaviours over the post-transport period. The video data were analysed to determine the proportions of cattle within each treatment group that were either eating/drinking, lying or standing over the initial 6 h post-transport period.

2.5 Statistical Analysis

Several blood parameters were transformed to stabilise variances prior to the analysis. The pre-transport measurements were analysed using the GLM procedure in SAS (SAS Institute Inc., Cary, NC, USA.). The initial model contained the fixed effects of transport duration (duration), week of transport (week) and their interaction. The post-transport measurements were analysed using the Mixed model procedure in SAS. The initial model contained the fixed effects of transport duration, week of transport and sampling time post-transport (Time) and their interactions plus a random term for animal. Non-significant interactions were sequentially removed to reveal the final model. A chi-

square test was used to analyse the behavioural data. Specifically, differences between duration treatments in the proportions of time spent lying or standing during the initial 3 h and second 3 h post-transport periods were examined.

3. Results

3.1 Body temperature

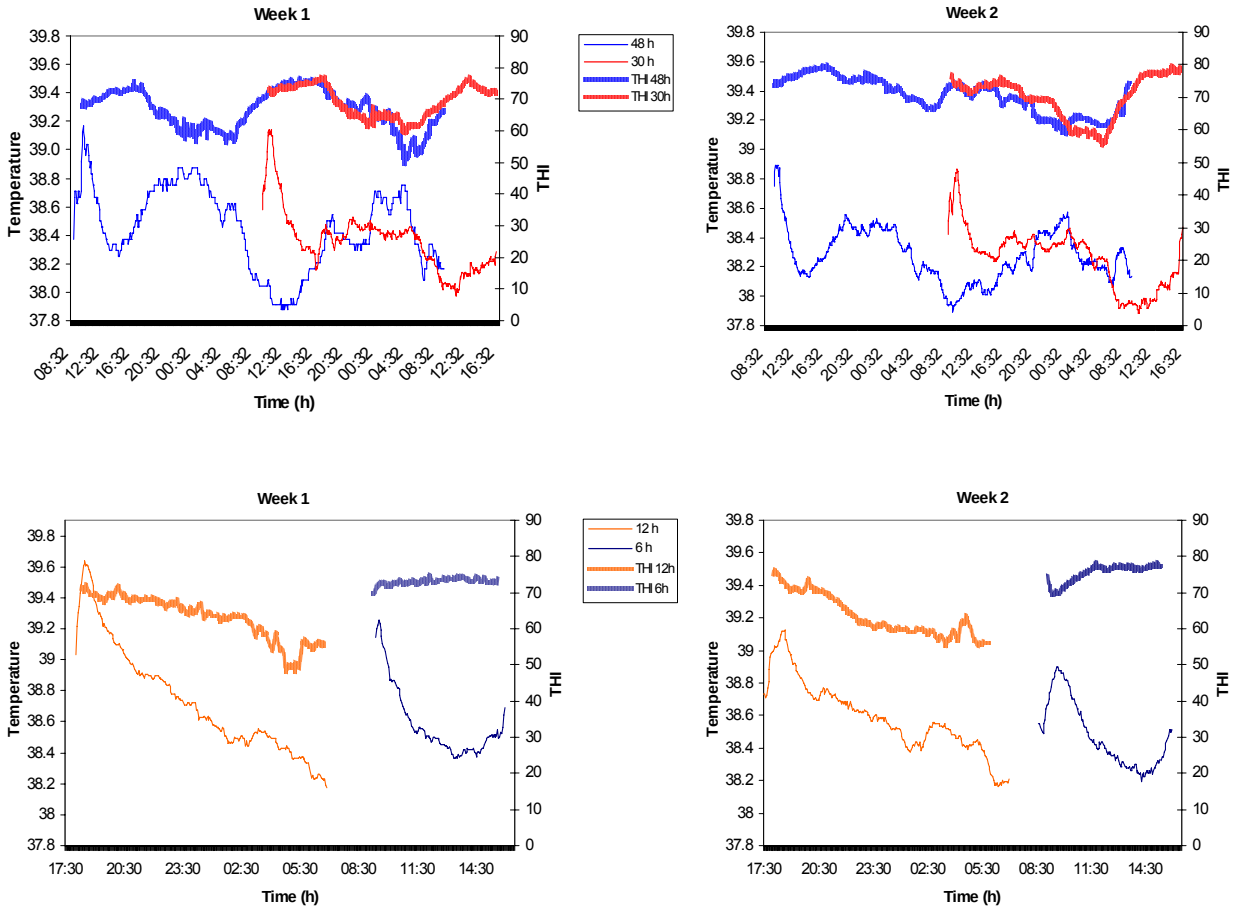


Figure 1 Mean rectal temperature profiles and on-board THI for the four journey durations/week

The combination of loading and initial stages of transport were the most stressful as indicated by the sharp rise in rectal temperature at the beginning of each profile. The increase in temperature at the conclusion of the two 6 h journeys and the 30 h journey in week 2 was associated with the unloading of the animals. In each case the temperature increase was lower than that observed on loading. For the longer journey durations of 30 and 48 h, a diurnal temperature pattern was evident. This pattern was in concert with changes in THI on-board the truck. The on-board conditions could best be described as moderate based on the general range in THI. In the main however, the temperature

profiles indicate that the cattle have generally habituated to the journey conditions after about 3-4 h from departure.

3.2 Blood chemistry

The pre-transport values of the blood chemistry variables generally conformed to accepted normal ranges (Table 1). Differences in pre-transport values between transport durations and week replicates are presented in Table 2. Although there were some pre-transport differences, the pre-transport value was generally not a significant covariate in the model analysing effects on post–transport values, and was removed. The effects of transport duration and recovery time post-transport on blood chemistry variables are presented in Table 3.

Table 1 Means and ranges for pre-transport blood parameters

Blood Parameter	Mean	Range
Blood urea nitrogen (BUN mg/dL)	14.15	1.0 – 20.0
Total protein (TP g/L)	71.90	60.0 – 85.0
Albumin (Alb. g/L)	38.04	31.4 – 53.0
Creatine kinase (CK U/L)	291.20	53.0 – 1783.0
β-hydroxy butyrate (BHB mmol/L)	0.25	0.02 – 0.72
Cortisol (nmol/L)	128.37	5.5 – 288.8
Osmolality (Osmol. mOsmol/L)	296.36	266.0 – 329.0
Haemoglobin (HGB g/L)	146.21	117.0 – 174.0
Red cell count (RCC x 10^{12}/L)	9.26	6.5 – 12.1
Haematocrit (HCT %)	0.42	0.32 – 0.52
Mean corpuscular vol. (MCV fL)	45.68	37.0 – 57.0
Mean conc. HGB in red cells (MCHC g/L)	348.17	316.0 – 370.0
Mean corpuscular HGB (MCH %)	15.93	13.0 – 20.0
Platelets count (PC x 10^9/L)	311.15	37.0 – 891.0
White blood cell count(WCC x 10^9/L)	6.82	3.6 – 12.9
Neutrophils (NEU x 10^9/L)	3.40	1.4 – 9.0
Lymphocytes (LYM x 10^9/L)	2.36	0.8 – 6.4
Mononcytes (MON x 10^9/L)	0.53	0 – 2.0
Eosinophils (EOS x 10^9/L)	0.48	0 – 1.5
Basophils (BAS x 10^9/L)	0.05	0 – 0.2

Table 2 Adjusted LS means for pre-transport plasma concentrations of blood urea nitrogen (BUN mg/dL), total protein (TP), albumin (Alb), creatine kinase (CK), β-hydroxy butyrate (BHB), cortisol (Cort) and osmolality (Osmol)

Main Effects	**BUN**	**TP**	**Alb***	**CK***	**BHB***	**Cort**	**Osmol**
Duration							
6 h	13.47	70.2	37.79	163.46	0.21	117.16	293.43
12 h	14.30	72.93	37.96	162.24	0.20	104.78	301.20
30 h	13.43	71.01	37.36	194.70	0.27	116.90	294.37
48 h	15.40	73.43	38.64	275.33	0.27	141.39	296.43
SED	0.65	1.18	0.02	0.006	0.03	0.72	2.60
Significance	*P<0.001*	*P<0.05*	*ns*	*P<0.01*	*P<0.05*	*ns*	*P<0.05*
Week							
1	14.38	71.65	38.57	198.54	0.24	141.32	297.82
2	13.92	72.14	37.30	187.33	0.24	99.80	294.92
SED	0.46	0.84	0.01	0.004	0.02	0.51	1.84
Significance	*ns*	*ns*	*P<0.01*	*ns*	*ns*	*P<0.001*	*ns*
Duration x Week							
6h x week 1			40.03				297.33
12 h x week1			35.64				289.53
30 h x week 1			37.91				301.87
48 h x week 1			38.01				300.53
6h x week 2			37.77				291.33
12 h x week 2			36.95				297.40
30 h x week 2			38.59				300.73
48 h x week 2			38.70				292.13
SED			0.02				3.67
Significance	*ns*	*ns*	*P<0.01*	*ns*	*ns*	*ns*	*P<0.05*

*backtransformed least square means shown

Table 3 Effect of transport duration, week of transport and time post-transport on plasma concentrations of blood urea nitrogen (BUN), total protein (TP), albumin (Alb), creatine kinase (CK), β-hydroxy butyrate (BHB), cortisol (Cort) and osmolality (Osmol)

Main Effects	BUN	TP	Alb*	CK*	BHB*	Cort	Osmol
Duration							
6 h	12.25	73.51	38.08	265.67	0.17	76.80	295.90
12 h	12.52	73.85	37.94	325.78	0.16	79.84	297.00
30 h	12.61	74.41	38.45	374.12	0.12	72.44	297.71
48 h	13.11	74.01	38.32	397.68	0.17	86.23	298.27
SED	0.45	1.03	0.01	0.006	0.019	0.44	1.26
Significance	*ns*	*ns*	*ns*	*ns*	*P<0.01*	*ns*	*ns*
Week							
1	14.38	73.72	38.56	334.81	0.17	91.40	297.28
2	13.92	74.17	37.80	336.10	0.14	67.04	297.15
SED	0.326	0.73	0.009	0.004	0.013	0.31	0.89
Significance	*P<0.001*	*ns*	*P<0.05*	*ns*	*P<0.01*	*P<0.001*	*ns*
Time post-transport							
0 h (arrival)	16.34	76.05	39.33	693.98	0.16	71.99	301.12
24 h	10.51	72.43	37.60	393.34	0.16	84.05	297.81
72 h	11.02	73.37	37.67	165.89	0.15	80.45	297.72
SED	0.31	0.52	0.006	0.002	0.014	0.20	0.91
Significance	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.001*	*ns*	*P<0.01*	*P<0.001*
Interactions							
Dur. x Week	*P<0.05*	*ns*	*ns*	*P=0.06*	*ns*	*ns*	*P<0.01*
Dur. x time	*P<0.001*	*P<0.01*	*P<0.05*	*ns*	*P<0.01*	*P<0.001*	*P<0.001*
Week x Time	*P<0.001*	*P<0.001*	*P<0.01*	*ns*	*ns*	*ns*	*P<0.001*
Dur. x Week x Time	*P<0.001*	*P<0.01*	*P<0.05*	*ns*	*P<0.01*	*ns*	*P<0.001*

*backtransformed means shown

For blood urea nitrogen concentrations, values were elevated immediately post-transport, compared with samples taken 24 and 72 h post-transport. There was a significant effect of transport duration × week × time, in which the animals transported for 48 h during week 2 recorded the highest blood urea nitrogen concentrations immediately after transport (Figure 2). The other blood chemistry indicators of hydration, total protein and albumin, exhibited similar patterns, with a decrease in concentrations during the first 24 h post transport (Table 3), and a transport duration × week × time interaction (Figure 2).

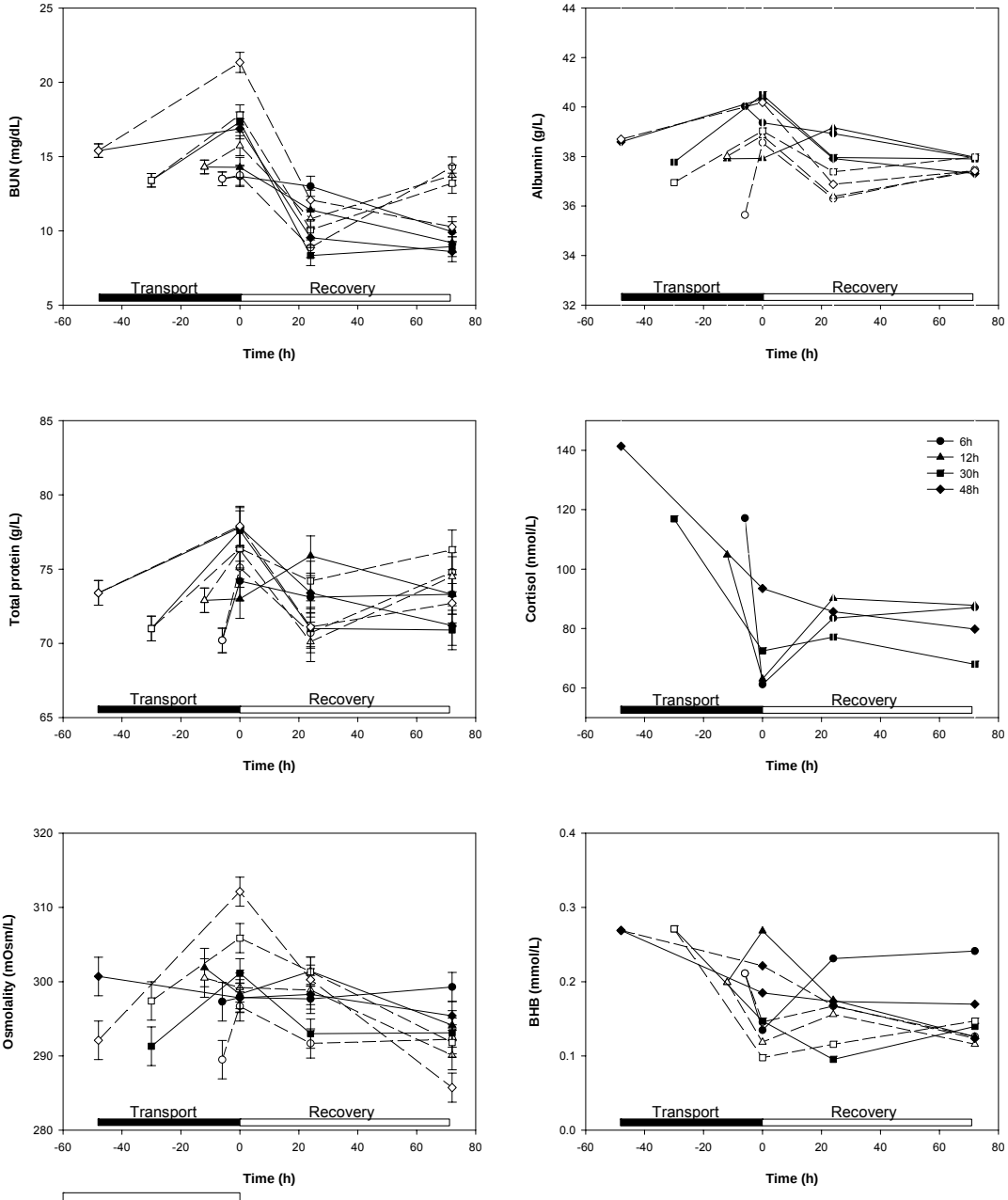

BUN (mg/dL)
Albumin (g/L)
Total protein (g/L)
Cortisol (nmol/L)
Osmolality (mOsm/L)
BHB (mmol/L)
Time (h)
Transport
Recovery
6h
12h
30h
48h
6 h wk1
6 h wk2
12 h wk1
12 h wk2
30 h wk1
30 h wk2
48 h wk1
48 h wk2

Figure 2 Changes in plasma BUN, albumin, total protein, cortisol, osmolality and β-hydroxy butyrate concentrations (LS means) following different transport durations and during 72 h of recovery.

Although blood concentrations of the muscle enzyme CK were increased following transport and then progressively declined during the 72-h recovery period, there were no effects of transport duration. Concentrations of β-hydroxy butyrate did not exhibit a consistent pattern and were not substantially increased by transport. Plasma cortisol concentrations exhibited a significant transport duration × time effect, whereby immediately post-transport, the highest cortisol concentrations were recorded in animals transported for 48 h, followed by animals transported for 30 h, with animals from the 6- and 12-h treatments lower again and not different from each other (Figure 2).

3.2 Haematology

The pre-transport values for blood cell variables are presented in Table 4. Among the key variables, only neutrophil counts differed between animals assigned to the different transport duration treatments when sampled pre-transport. Post-transport haematology results are presented in Table 5.

White blood cell counts were increased by transport, and then declined over the 72-h recovery period. However, an effect of transport duration × time revealed that the white blood cell counts immediately post-transport were highest for animals transported for 6 h, followed by animals on the 12, 30 and 48-h treatments, respectively (Figure 3). Within the white cell populations, neutrophil numbers were increased by transport and then declined over the 72-h recovery period. The greatest neutrophil counts immediately post-transport were recorded for the 6-h transport treatments (Figure 3). Lymphocyte counts post-transport did not exhibit a consistent pattern (Table 5, Figure 3).

For red blood cell counts, a significant transport duration × time effect was found (Figure 3), in which the decline in red blood cell numbers during the first 24 h post-transport was more pronounced for animals transported for 30 and 48 h. The haematocrit values were not substantially increased by transport, and although there was a slight decline between 0 and 24 h post-transport, values stabilised (week 1) or increased slightly (week 2) at the 72-h sampling time point (Figure 3).

Table 4 Adjusted LS means for pre-transport haematology parameters

Main Effects	HGB	RCC	HCT	MCV	MCH	MCHC	PC	WBC*	NEU*	LYM*	MONO*	EOS*	BAS*
Duration (D)													
6 h	147.71	9.37	0.42	45.35	15.86	350.36	379.99	6.63	3.06	2.44	0.56	0.46	0.065
12 h	144.13	9.35	0.42	45.20	15.67	344.97	245.30	6.48	3.01	2.37	0.51	0.38	0.049
30 h	143.67	9.06	0.42	45.97	15.97	347.00	336.17	6.41	3.21	2.12	0.49	0.47	0.053
48 h	149.43	9.28	0.43	46.23	16.23	350.23	285.50	7.08	3.93	2.11	0.52	0.43	0.048
SED	2.72	0.27	0.008	1.03	0.35	1.81	48.69	0.06	0.44	0.05	0.02	0.03	0.002
Significance	*ns*	*ns*	*ns*	*ns*	*ns*	*P<0.01*	*P<0.05*	*ns*	*P<0.001*	*ns*	*ns*	*ns*	*ns*
Week (Wk)													
1	144.78	9.17	0.41	44.52	15.92	356.55	310.15	6.78	3.27	2.36	0.55	0.42	0.055
2	147.69	9.36	0.44	46.86	15.95	339.73	313.33	6.51	3.31	2.16	0.49	0.45	0.053
SED	1.92	0.19	0.006	0.73	0.25	1.28	34.29	0.04	0.03	0.04	0.01	0.02	0.001
Significance	*ns*	*ns*	*P<0.001*	*P<0.001*	*ns*	*P<0.001*	*ns*	*ns*	*ns*	*ns*	*P=0.06*	*ns*	*ns*
D x Wk													
6 h x wk 1			0.42			355.93			3.18	2.25			0.052
12 h x wk 1			0.39			358.73			2.32	3.09			0.044
30 h x wk 1			0.40			357.00			3.26	2.15			0.055
48 h x wk 1			0.42			354.53			4.54	2.01			0.067
6 h x wk 2			0.43			344.79			2.93	2.63			0.079
12 h x wk 2			0.45			331.20			3.81	1.74			0.054
30 h x wk 2			0.43			337.00			3.16	2.09			0.052
48 h x wk 2			0.43			345.93			3.37	2.22			0.029
SED			0.012			2.58			0.06	0.08			0.003
Significance	*ns*	*ns*	*P<0.001*	*ns*	*ns*	*P<0.001*	*ns*	*ns*	*P<0.001*	*ns*	*ns*	*ns*	*P<0.05*

*backtransformed least square means shown

Table 5 Effect of transport duration, week of transport and time post-transport on blood haematology

Main Effects	HGB	RCC	HCT	MCV	MCH	MCHC	PC	WBC*	NEU*	LYM*	MONO*	EOS*	BAS*
Duration (D)													
6 h	148.58	9.39	0.44	46.98	16.02	339.56	455.38	6.95	4.10	3.11	0.65	0.77	0.034
12 h	143.88	9.27	0.42	45.62	15.77	345.26	451.44	5.45	4.18	2.21	0.60	0.66	0.044
30 h	144.18	9.05	0.42	47.01	16.08	341.47	485.54	5.46	2.58	3.54	0.61	0.80	0.072
48 h	145.81	9.08	0.43	47.58	16.20	340.86	432.82	4.22	2.85	2.16	0.61	0.78	0.051
SED	2.60	0.25	0.008	1.02	0.36	1.46	35.96	0.05	0.06	0.06	) 0.01	0.029	0.002
Significance	*ns*	*ns*	*P=0.05*	*ns*	*ns*	*P<0.01*	*ns*	*P<0.001*	*P<0.001*	*P<0.001*	*ns*	*P<0.05*	*P<0.001*
Week (Wk)													
1	144..89	9.13	0.41	45.45	16.05	352.15	426.34	5.64	3.31	2.89	0.67	0.77	0.042
2	146.33	9.26	0.44	48.14	15.99	331.42	453.92	5.29	3.46	2.56	0.57	0.73	0.059
SED	1.84	0.18	0.005	0.72	0.26	1.04	25.42	0.04	0.04	0.04	0.009	0.02	0.01
Significance	*ns*	*ns*	*P<0.001*	*P<0.001*	*ns*	*P<0.001*	*P<0.05*	*ns*	*ns*	*P=0.08*	*P<0.01*	*ns*	*ns*
Time (T)													
0 h (arrival)	150.29	9.57	0.44	45.82	15.87	345.94	453.92	7.74	5.60	2.79	0.67	0.55	0.042
24 h	141.99	8.94	0.41	46.63	16.11	343.85	468.03	5.40	2.98	2.86	0.59	1.26	0.039
72 h	144.55	9.08	0.43	47.93	16.08	335.57	446.94	3.76	2.12	2.52	0.60	0.67	0.070
SED		0.045	0.002	0.09	0.05	0.84	14.39	0.02	0.09	0.02	0.008	0.014	0.001
Significance		*P<0.001*	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.001*	*ns*	*P<0.001*	*P<0.001*	*P<0.01*	*P<0.001*	*P<0.001*	*P<0.001*
Interactions	*ns*	*ns*	*P=0.07*	*ns*	*ns*	*P<0.001*	*P<0.01*	*ns*	*P<0.05*	*ns*	*P<0.05*	*ns*	*ns*
D x Wk	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.001*	*ns*	*P<0.001*	*P<0.05*	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.001*
D x T	*P<0.001*	*P<0.05*	*P<0.05*	*ns*	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.05*	*P<0.01*	*P<0.001*
Wk x T	*P<0.01*	*ns*	*P<0.001*	*P<0.001*	*ns*	*P<0.001*	*P<0.05*	*ns*	*P<0.001*	*P<0.01*	*ns*	*ns*	*ns*
D x Wk x T													

*backtransformed least square means shown

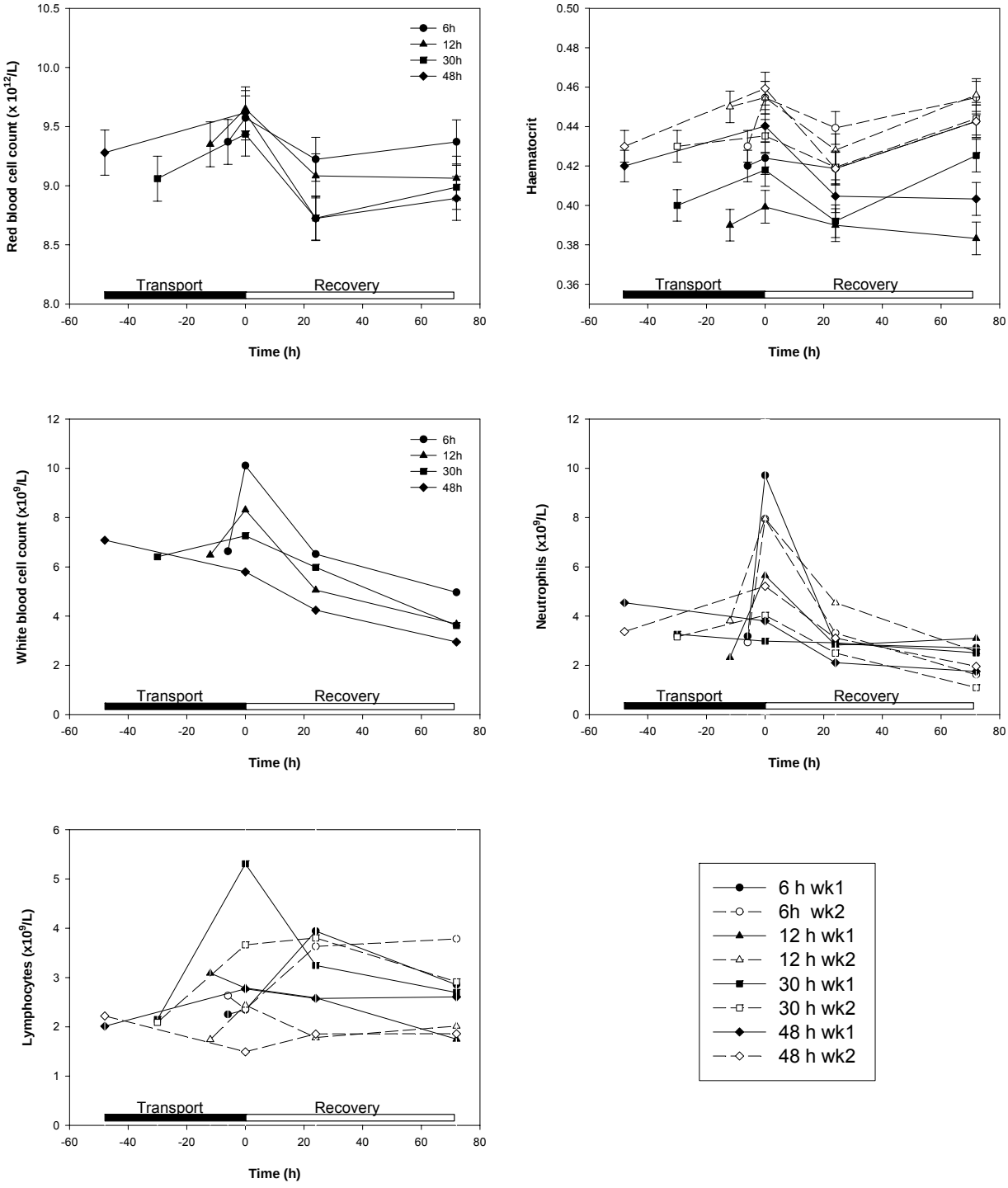


Figure 3 Changes in selected haematology parameters (LS means) following different transport durations and during 72 h of recovery

3.3 Liveweight

Due to the treatment allocation process, there were no differences in pre-transport liveweight between treatments. A significant interaction between transport duration x week x time was observed for liveweight (P<0.01) (Table 6, Figure 4). The greatest variation in liveweight between the transport duration treatments was observed on arrival. The cattle lost on average 20.1, 22.3, 25.4 and 37.8 kg after being transported 6, 12, 30 and 48 h, respectively. However, the differences in liveweight between the transport treatments on arrival were only significant for the contrast between the 48 h (week 2) and 6 h (weeks 1 and 2), 12 h (week 1 and 2) and 30 h (week 2) treatment groups. During the 72 h recovery period, the majority (95-98%) of the liveweight lost was recovered. Transport duration did not affect ADG during the 43-d feedlot period (Table 6).

Table 6 Effect of transport duration and week of transport on feedlot average daily gain (ADG) and transport duration x week of transport x time post-transport on post-transport liveweight

Main Effects	Post-transport liveweight (kg)	Feedlot ADG (kg/day)
Duration (D)		
6 h	359.0	1.80
12 h	357.0	2.10
30 h	352.8	2.08
48 h	351.4	1.93
SED	5.2	0.14
Significance	*ns*	*ns*
Week (Wk)		
1	355.3	1.82
2	354.9	2.14
SED	3.7	0.09
Significance	*ns*	*P<0.01*
Time (T)		-
0 h (arrival)	345.8	
24 h	361.4	
72 h	358.0	
SED	1.0	
Significance	*P<0.001*	
Interactions		-
D x Wk	*ns*	
D x T	*P<0.001*	
Wk x T	*P<0.001*	
D x Wk x T	*P<0.01*	

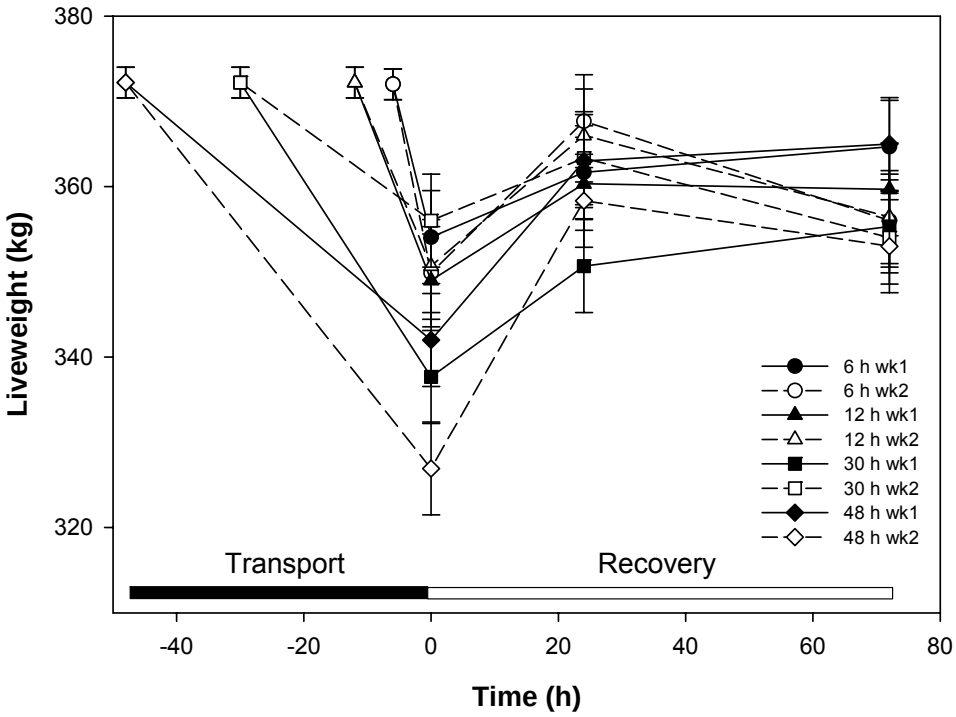


Figure 4 Changes in liveweight (LS means) following different transport durations and during 72 h of recovery

3.4 Post-transport behaviour

Significant differences in lying behaviour were found between two transport duration treatments in both weeks (Table 7, Figure 5)

Table 7 Differences in the time spent lying over a 6 h post-transport period after 12 and 48 hours of transport. Results are presented as min/animal, out of a total of 180 min for each period.

Post-transport period	Transport duration		Significance
	12 h	48 h	
Week 1			
0 – 3 h	29	132	P<0.001
3 – 6 h	74	77	ns
Week 2			
0 – 3 h	36	139	P<0.001
3 – 6 h	38	89	P<0.001

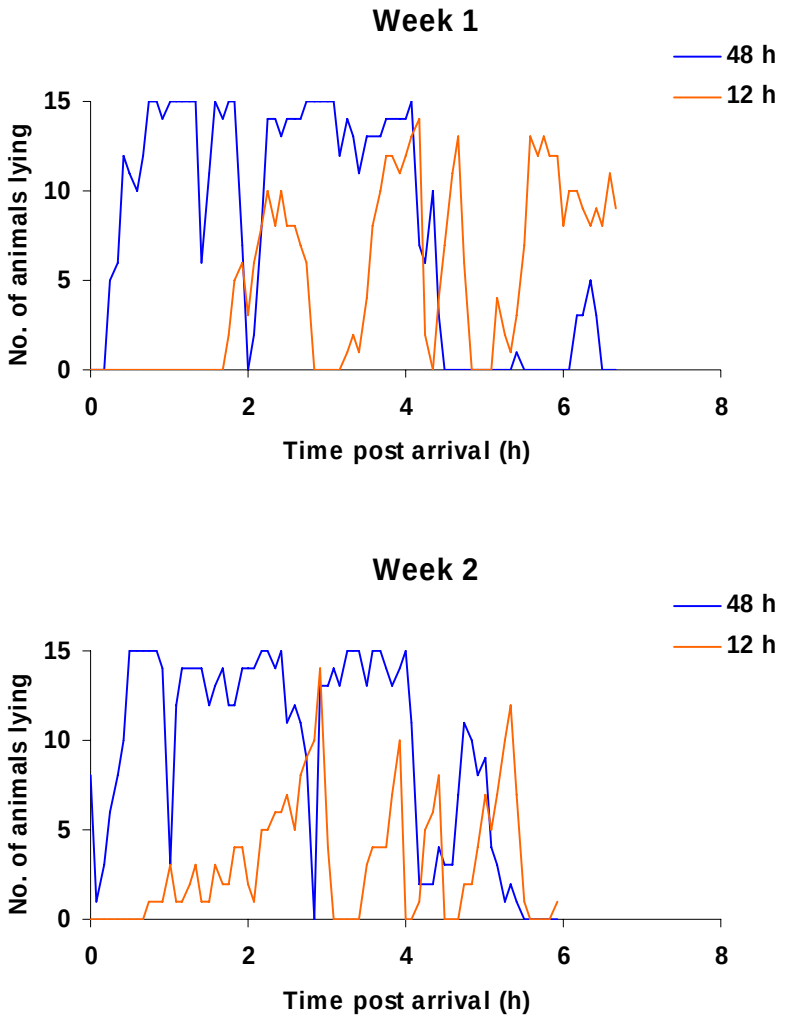


Figure 5 Proportions of cattle lying post-transport (over 6 h period) after 12 and 48 h of transport

Cattle transported for 48 h were clearly more tired given their preference to spend more time lying ($P<0.001$) during the initial 3 h post-transport recovery period compared with those transported for 12 h. The results during the 3 - 6 h post-transport period differed between weeks. In week 1, there was no difference between the treatments whereas in week 2, the cattle from the 12 h transport treatment spent significantly less time lying down ($P<0.001$). In general, the time spent lying over the two post-transport periods was reasonably similar between weeks for the 48 h transport cattle. The trend

particularly during the second 3 h post-transport period was less consistent between weeks for the 12 h transported cattle.

4. Discussion

The results of this study generally indicate that healthy cattle that have not had restricted access to food or water prior to transport can tolerate transport up to 48 h without any major compromise to their welfare. Some of the key physiological indicators were clearly influenced by transport duration however, the changes were not extreme and still within the normal expected physiological ranges for cattle (Kaneko et al 1997).

Of the plasma variables measured, we expected that transport duration would probably have the largest effect on those that were indicative of haemoconcentration and therefore, dehydration (eg. haematocrit, osmolality, total protein and albumin) and muscle use or damage (creatine kinase). In general, the plasma indicators of dehydration on arrival were affected by transport duration however, there were exceptions and differences between the weekly replicates. The concentration of plasma total protein and albumin after 30 h and 48 h of transport (albumin 39.0 – 40.5 g/L; total protein 76.4 – 77.9 g/L) were considerably lower than those observed by Knowles et al (1999) in response to 31 h transport (albumin 43.2 g/L; total protein 86.3 g/L). In contrast, the osmolality values on arrival after 30 or 48 h (301.1 – 312.1 mOsm/L) were higher than the level (293.6 mOsm/L) observed by Knowles et al (1999). In the study by Knowles et al (1999), cattle had unrestricted access to food and water prior to transport. The main difference between the studies was that their cattle were rested for 1 h with access to water on-board the trucks after 14 h of the journey. Whilst the authors reported that not every animal drank, it is interesting to note that the changes in plasma total protein and albumin would suggest more apparent dehydration in their study. In the present study, haemoconcentration associated with increasing journey duration was apparent, but the level of dehydration even after 48 h could not be classed as being of clinical concern. This outcome can largely be attributed to the ruminal reservoir of fluid which acts as a useful buffer during periods of water restriction (Knowles and Warriss 2000). Another factor could be the role the HPA axis and specifically, cortisol plays in the maintenance of water balance through its suppression of the renin-angiotensin-aldosterone axis (Parker et al 2004). The important caveat here is that the results observed in the current study may not hold true when pre-transport water curfews are applied before journeys of similar duration.

Creatine kinase levels increased with increasing transport duration however, these differences were not significant. 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) reported that plasma levels of creatine kinase were positively associated with journey length of 5-15 h. Knowles et al (1999) observed that after an initial increase in plasma creatine kinase after 14 h of transport, the concentrations remained at similar levels for longer journeys between 14 – 31 h. The inter-animal variability is perhaps the primary reason for a lack of a significant effect in the present study, and suggests that the CK concentrations measured were more indicative of individual animal events (e.g. muscle

trauma or bruising), rather than an increasing muscle exhaustion associated with transport duration.

The plasma measure of protein catabolism (blood urea nitrogen) increased commensurate with transport duration but once again the levels attained even after 48 h transport were well with the normal range for cattle. The decrease in the ketone β-hydroxy butyrate which is indicative of lipid catabolism in response to transport was similar to that observed by Knowles et al (1999). The results in both studies were unexpected given that fasting generally causes an increase β-hydroxy butyrate as lipid is mobilised for energy. Knowles et al (1999) attributed the decrease to the combined effects of the journey and the change in diet prior to transport.

The trends in the rectal temperature profiles reinforce the general view that loading and the initial stages of transport are the most stressful (Eldridge et al 1988, Warriss et al 1995, Broom et al 1996, Pettiford et al 2006). After 3-4 h of transport, rectal temperatures returned and remained at normal levels irrespective of journey duration, suggesting that the cattle had habituated to the journey conditions.

The haematology results showed that there was a neutrophilia associated with the shorter transport durations. It is likely that this is related to the stress response seen during the initial stages of transport (Pettiford et al 2006), and that the same effect occurred for animals on the longer duration treatments, but was resolved by the time of post-transport sampling. 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.

Although transport resulted in significantly decreased body weights immediately post-journey, there was either no or a minimal effect of transport duration on the amount of weight lost during transport or the recovery during the 72 h post-transport period. The body weight results suggest that the weight loss caused by transport was essentially related to the loss of gastrointestinal contents, and that animals on the longer-duration treatments suffered no problems in realimentation during the period after arrival. The different transport durations and therefore concomitant periods of fasting did not affect feedlot ADG. This tends to confirm the results of Robbins et al (1982) who compared different fasting treatments (0, 32 and 56 h) and showed that there were no permanent effects on subsequent feedlot growth for 100 days.

The post-transport behavioural data clearly indicates that cattle transported for 48 h compared to 12 h were more fatigued given their desire to spend more time lying during the initial 3 h recovery period. However, this trend was less evident during the second 3 h post-transport period suggesting that cattle transported for 48 h had recovered sufficiently after 3 h to behave in a similar manner to those transported over a considerably shorter duration.

Finally, it is pertinent to highlight that the differences between the replicates (ie week) was a large source of variation for many of the physiological parameters. This reinforces

the need for replication in animal studies but also flags a cautionary note when drawing conclusions based on a single cohort of animals.

5. Conclusions

Healthy mature cattle with no pre-transport feed or water curfew and transported in accordance with accepted good practice generally coped with transport durations up to 48 h. Whilst those on the longer journeys (especially 48 h), were more tired, the physiological data indicates that they were not clinically compromised. Given these results, the current maximum duration of 36 h with the option to extend to 48 h (if the animals are not displaying obvious signs of fatigue, thirst or distress and if the extension allows the journey to be completed within 48 h) under the Australian Model Code of Practice for the Land Transportation of Cattle, is acceptable on animal welfare grounds for the class of animal examined in this study.

6. References

Broom, D.M., Goode, J.A., Hall, S.J.G., Lloyd, D.M and Parrott, R.F. (1996). Hormonal and physiological effects of a 15 hour road journey in sheep: comparison with the responses to loading, handling and penning in the absence of transport. *Br. Vet. J.* **152**:593-605.

Burrow, H.M., Colditz, I.G and Oddy, V.H. (2005). Relationships between temperament and haematological values and the effects of an electrolyte replacement treatment on weight loss during, and recovery following, long distance transport of *Bos indicus* and Zebu derived weaner cattle. *Aust. J. Agric. Res*. (submitted).

Eldridge, G.A., Winfield, C.G. and Cahill, D.J. (1988). Responses of cattle to different space allowances, pen sizes and road conditions during transport. *Aust. J. Exper. Agric.* **28**:155-159.

Jones, G.E. and Mould, D.L. (1984). Adaptation of the guaiacol (peroxidase) test for haptoglobins in a microtitration plate system. *Res. Vet. Sci*. **37**:87-92.

Knowles, T.G. and Wariss, P.D. (2000). Stress physiology of animals during transport. In: "Livestock Handling and Transport" 2nd Edition. Ed T.Grandin, (CAB International, Oxon UK).

Kaneoko, J.J., Harvey, J.W. and Bruss, M.L. Eds. (1997). "Clinical Biochemistry of Domestic Animals" 5th Edition. (Academic Press, London, UK).

Parker, A.J., Hamlin, G.P., Coleman, C.J. and Fitzpatrick, L.A. (2004). Excess cortisol interferes with a principal mechanism of resistance to dehydration in *Bos indicus* steers. *J. Anim. Sci*. **82**: 1037-1045

Pettiford, S.G., Ferguson, D.M., Lea, J.M., Lee, C., Paull, D., Reed, M.T. and Fisher, A.D. (2006). The effect of loading practices and 6 h road transport on the physiological responses of yearling cattle. *J. Anim. Sci*. (in preparation).

Slocombe, L.L. and Colditz, I.G. (2005). Evaluating the stress of production in cattle using haptoglobin. 5th Annual International Colloquium on Animal Acute Phase Proteins, Dublin, Ireland.

Warriss, P.D., Brown, S.N., Knowles, T.G., Kestiin, S.C., Edwards, J.E., Dolan, S.K. and Phillips, A.J. (1995). Effects on cattle of transport by road for up to 15 hours. *The Vet. Rec.* **126**:319-323.

Robbins, G.B., Laing, A.R., Bushell, J.J. and Ash, A.J. (1982). Effect of selling system for store steers on liveweight and subsequent performance if the feedlot. *Proc. Aust. Soc. Anim. Prod*. 14:381-384.

Milestone 6

Milestone report on completion of stage 2 cattle research. Animal welfare outcomes determined post-transport for different curfew and transport duration combinations. Recommendations provided on best practice transport management for curfew and duration combinations

Abstract

The aim of this experiment was to quantify the effect of pre-transport food and water deprivation (curfew) on the response to transport in yearling cattle. Eighty four pasture fed yearling heifers were used for the experiment. A factorial design was used comprising three pre-transport food and water deprivation treatments of 0, 12 and 24 h and two transport duration treatments of 12 and 24 h, and these were replicated twice over a period of 4 weeks. At the conclusion of their transport treatments, the cattle were placed in recovery pens with access to hay and water for 72 h. Detailed measurements of liveweight, blood chemistry, haemataology, urine concentration, body temperature, lying behaviour and water intake during recovery were recorded pre- and post-curfew, pre-transport and 0, 24, 48 and 72 h post-transport. The interaction between curfew treatment x transport treatment x time (sampling) was significant for many of the blood and urine measures, liveweight and behaviour. In general, the combined periods of curfew and transport were additive for their effects particularly for liveweight loss and measures of hydration status (serum osmolality, urine specific gravity and osmolality) observed immediately post-transport. The incidence of lying behaviour during transport and the proportion of time lying during the initial period (0-3 h) of post-transport recovery were higher for cattle transported 24 h compared to 12 h. Furthermore, the incidence of lying behaviour in transit was higher in curfewed cattle, particularly those curfewed 24 h over the longer journey, than non-curfewed cattle. The results of this investigation indicate that subjecting healthy, grass-fed cattle to pre-transport periods of food and water deprivation (12 and 24 h compared with 0 h) prior to transport for 12 or 24 h did not enhance the capacity of the animals to cope with transport. The results also indicate that periods of food and water deprivation prior to transport are simply additive to transport-related periods of deprivation, and provide further evidence that healthy cattle under non-threatening environmental conditions can tolerate periods of up to 48 h of feed and water deprivation without undue compromise to their welfare.

Project objectives

1. Determine the contribution of handling, loading and initial transport processes on the stress responses of cattle to road transport.
2. Determine the animal welfare outcomes of yearling cattle transported under controlled conditions for 6, 12, 30 or 48 hours from farm to feedlot entry.
3. Determine the animal welfare outcomes of mature sheep (eg. live export trade) transported under controlled conditions for 12, 30 or 48 hours.
4. Utilize a readily-applicable subset of welfare measures to determine the typical welfare outcomes of yearling cattle and cast for age sheep transported under a range of commercial conditions.
5. Develop recommendations on best practice yearling cattle and cast for age sheep transport management for optimal welfare and productivity.

Success in achieving milestone

THE EFFECT OF PRE-TRANSPORT PERIODS (0, 12 AND 24 H) OF FOOD AND WATER DEPRIVATION ON THE RESPONSE TO 12 AND 24 H OF TRANPORT IN YEARLING HEIFERS

Drewe Ferguson, Bernadette Earley, Dominic Niemeyer, Caroline Lee, David Paull, Jim Lea, Matt Reed and Andrew Fisher

1. Introduction

The standards of husbandry and welfare practiced during livestock production are becoming important factors influencing consumer perceptions in many markets. Clearly, the use of welfare-unfriendly livestock transport practices has the potential to downgrade product quality. Furthermore, the use of practices that initiate market and public concerns that are unable to be adequately addressed may cause damage to the image and market access of Australian livestock products.

Unfortunately, the ability of Australian livestock industries to demonstrate the welfare status of livestock during land transport remains vulnerable on several grounds. There is little scientific evidence that the welfare of cattle and sheep trucked under Australian conditions is superior or equivalent to that of animals transported by our competitors and international trading partners. Further, there are some marked differences in our conditions and practices compared with those of others. If Australian practices and regulations are going to be different of necessity from those of other trading blocs, then we may need objective evidence that the animal welfare outcomes are equivalent and acceptable. Consequently this project was designed to develop scientifically defensible quantification of the animal welfare outcomes of Australian livestock transport practices. The research reported here represents the third in a series of investigations examining the animal welfare outcomes of land transport practices for cattle and sheep.

During transport, it is inevitable that there will be short to moderate periods of restricted access to food and water. Whilst food and water deprivation will normally occur during transport, the period of deprivation can be substantially extended if animals undergo a curfew prior to transport. Curfew is the generic term used in livestock industries for the practice of enforced food or food and water deprivation prior to transport, sale or slaughter. They are applied because of the demands and selling conditions by transport operators, livestock buyers and abattoir management, respectively in Australia. Curfews are typically 6 – 12 h in duration and the primary reason for their use is to reduce the gastrointestinal volume (empty out) prior to transport, thus reducing the total amount of excreta in trucks and the level of faecal soiling on animals.

Transport operators have advocated that pre-transport curfews enable the animals to travel better. One of the primary benefits observed was the reduction in number of animals (primarily cattle) that lie down on the truck during the journey. The risk of bruising and injury increases considerably when animals go down (Tarrant and Grandin 2000) and drivers are required to encourage these animals back to their feet which in turn, may cause additional stress in both the downed animal and others in the truck.

Regular stopping to attend to downed animals will also prolong the transport duration. One factor thought to contribute to the curfew mediated reduction in downer animals was the reduced volume of excreta on the truck floor and therefore reduced risk of slippage and falling.

There is very little published data corroborating these anecdotal views from the transport industry although it is clear that non-curfewed cattle will produce more excreta during transport (Gregory et al 2000) and it is also likely to be more liquid (Bass and Duganzich 1980). Whilst the volume of excreta and indeed the design and construction of the stockcrate floor contribute to losses of balance and slippage, it is pertinent to highlight that stocking density and driving events (eg. braking, cornering) are also major factors in this context (Eldridge 1988, Tarrant et al 1992, Cockram et al 2004).

No clear conclusions can be drawn with regard to the interaction between pre-transport food and water deprivation and the response to transport, particularly to long-haul transport, as there is a paucity of published data. The research by Irish workers (Earley et al 2004) sheds some light on this interaction when cattle were transported over a moderate duration. Earley et al (2004) contrasted the treatments of 8 h of fasting (with access to water) versus no fasting on the responses to 8 h of road transport. Apart from a difference in liveweight lost after transport (9.4% fasted and 7.2% non-fasted), there were no or minimal differences in blood chemistry and haematology. Given this, they concluded that the combination of 8h of fasting and 8 h of transport did not negatively impact on animal welfare.

This investigation was undertaken in view of the lack of scientific data to support the anecdotal views from livestock transporters that periods of food and water deprivation prior to loading improve the capacity of cattle and sheep to cope with transport.

2. Materials and Methods

The experiment was approved by the CSIRO Livestock Industries FD McMaster Animal Ethics Committee (Approval No. 06/13).

2.1 Cattle

Eighty-four Beefmaker yearling heifers were sourced from a local property in the New England region for this experiment. The cattle were transported to the FD McMaster Laboratory two months before the commencement of the study where they were maintained on improved pastured paddocks. Fourteen days before the commencement of the experiment, the cattle were allocated to their six food+water deprivation x transport treatments stratified for liveweight. These were randomly allocated to four groups of 21 head. The average liveweight of the group prior to the commencement of the transport treatments was 261.7 ± 12.4 kg.

2.2 Experimental design and procedures

A factorial design was used comprising three pre-transport food and water deprivation treatments of 0, 12 and 24 h and two transport duration treatments of 12 and 24 h, and these were replicated twice over a period of 4 weeks. The cattle were transported on the CSIRO stock truck (16 t fixed chassis vehicle) over a route that included highway and

secondary roads. The journey length was approximately 5.5 h in duration. This journey was repeated twice and four times for the 12 and 24 h journey duration treatments, respectively. Drivers either changed over or took a 30-min break between journey runs. During this time the cattle remained on the vehicle.
The experimental schedule for each week is shown in Appendix 1. On each week, three curfew/transport treatment groups were trucked. At approximately 1.5 h prior to the commencement of the food+water deprivation treatments, the cattle were yarded. The seven treatment animals were drafted off and the remainder were returned to their home paddock. The seven cattle were weighed, fitted with rectal temperature loggers, IceTag^{TM} behavioural monitors and sampled for urine and blood. The cattle were then placed in a yard to commence their food+water deprivation treatment.

At the conclusion of their food+water deprivation period, the cattle were reweighed and further blood and urine samples were taken before the animals were loaded onto the truck to commence their transport duration tretaments. The animals were placed in the front pen of the stock crate at a stocking density of 0.82 m^2/head which complied with the welfare transport code for this liveweight class of cattle (Figure 1). The vehicle was fitted with a GPS logger and a temperature and humidity logger (Tiny Tag plus TGP1500 loggers, Omni Instruments, Scotland - temperature range -30 to +50^oC and relative humidity 0 to 100%) was placed in the stockcrate.

Figure 1 Cattle in stock crate

After completing their journeys, the cattle were unloaded at second, unfamiliar set of yards to commence their 72 h recovery period. Upon arrival, the cattle were weighed and blood and urine were collected. This was repeated 24, 48 and 72 h after arrival. During the 72-h recovery period, the cattle were placed in pens (15 x 6 m) with access to water and good quality hay as roughage. The water supply to each pen was monitored (Hobo® Event logger, Onset Computer Corporation, Bourne, MA 02535, USA) to measure the water utilisation by each group during the recovery phase.

After the 72-h recovery period, the IceTag^{TM} behavioural monitors and rectal temperature loggers were removed and the animals were placed in a new paddock.

2.3 Blood sampling and biochemical measurements

Each animal was blood sampled via jugular venipuncture on 6 occasions; prior to and on completion of the food+water deprivation period and 0, 24, 48 and 72 h post-transport. The sample taken on completion of the food+water deprivation treatment was also defined as the pre-transport sample. Two blood samples were taken at each time point (6ml EDTA and 10 ml serum vacutainers). An aliquot of whole blood was analysed for the haematology variables haemoglobin (HGB), red cell counts (RCC), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelet count (PC), white blood cells count (WBC) and differential cell counts of neutrophils (NEU), lymphocytes (LYM), monocytes (MON), eosonophils (EOS), basophils (BAS), using a Cell-Dyn Haematology Analyser 3500R (Abbott Diagnostics, CA, USA).

An aliquot of blood was centrifuged and the resultant serum was harvested and frozen (-20^{o}C) for subsequent analysis of blood chemistry.

The concentrations of cortisol, blood urea nitrogen, total protein, albumin, creatine kinase, β-hydroxy butyrate and haptoglobin were determined on the serum samples. Cortisol was determined using a Spectria Cortisol RIA (Orion Dianostica, Espoo, FIN). A OLYMPUS AU400 automated clinical was used to analyse the blood urea nitrogen, creatine kinase and total protein plasma concentrations. Osmolality was also measured, using a vapour pressure osmometer (Wescor, 5500). The acute phase protein haptoglobin was determined in plasma by a variation of the method of Jones and Mould (1984). The assay was modified to account for the effect of free haemoglobin due to haemolysis (Slocombe and Colditz, 2005).

2.4 Urine sampling and biochemical measurements

Urine was collected in sterile 50-ml containers whilst the animals were in the race or forcing yard, or if this was not possible, the animals were restrained and the urine was collected via insertion of a urinary catheter into the bladder. A small amount of Xylocaine gel was placed on the tip of the catheter to minimise discomfort upon insertion in the urethra. A 10 ml syringe was placed on the end of the catheter and this was used to withdraw 1 - 3 ml of urine from the bladder.

Urine specific gravity was measured with a veterinary refractometer (DLC Australia Pty Ltd, Caboolture, Qld, 4510 AUS). The urine was then frozen (20°C) and later analysed for osmolality.

2.5 Body temperature

On the day of transport, focal cattle were fitted with rectal temperature loggers. Temperature loggers (Thermochron iButton, Maxim Integrated Products, USA) were secured to probes that were placed in the rectum and secured in place to the tail head via surgical tape. The instrumentation allowed normal defaecation and movement of the tail. Body temperature was recorded every 4 min until the harnesses and probes were removed at 72 h post-transport.

2.6 Behavioural assessment

IceTag™ behavioural monitors (IceRobotics, Midlothian, Scotland, UK) were secured to the left hind leg of each animal via velcro and duct tape according to the manufacturer's instructions (Figure 2ab). These monitors recorded whether the animal was lying, standing or active (walking, moving) and measurements were recorded every second.

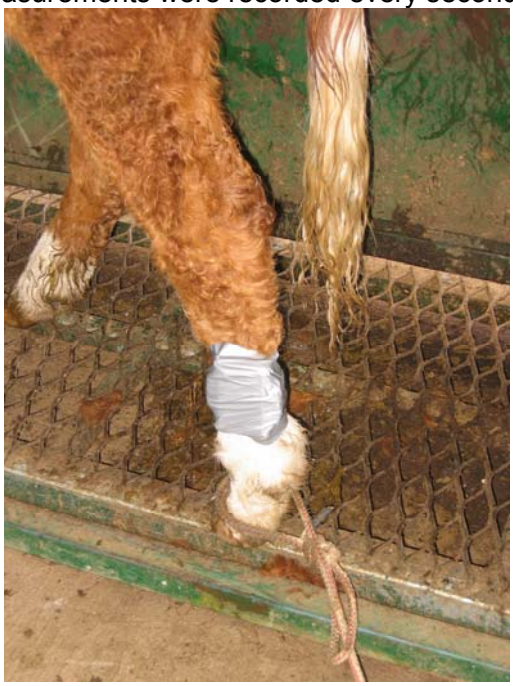
a.  b. 

Figure 2ab Fitting of IceTag™ behavioural monitor to left hind leg.

2.5 Statistical Analysis

Several blood parameters were transformed to stabilise variances prior to analysis. The MIXED model procedure in SAS (SAS Institute Inc., Cary, NC, USA.) was used to analyse the data. In determining the effects of curfew duration, the initial model contained the fixed effects of curfew treatment, replicate and time (curfew start and end) plus their interactions plus a random term for animal. A model comprising the terms curfew treatment, transport treatment, replicate and time (pre-transport, 0 h post-transport, 24 h post-transport, 48 h post-transport and 72h post-transport) and

interactions plus a random term for animal was used to quantify the main fixed effects of curfew and transport duration. These models were applied for the liveweight, blood and urine measurements. Lying behaviour during post-transport recovery was also analysed used the MIXED model procedure. Two analyses were conducted. The first analysis examined the changes in lying behaviour over the initial 24 h (8 periods of 3 h) of recovery. The second analysis examined these changes over the three consecutive 24-h periods of recovery. The terms in the model were similar to those described above with the exception that recovery period was included instead of sampling time. The water consumption during the initial 24 h of recovery and over the three 24-h periods of recovery was analysed using a repeated measures analysis using the GLM procedure in SAS. For all the analyses, non-significant interactions were sequentially removed to reveal the final model.

3. Results

3.1 Effect of curfew duration on physiological measures

For the majority of measurements, a significant three-way interaction between curfew treatment x time x replicate was observed, indicating that the effect of curfew duration on the change in these measures differed between replicates (Tables 1 and 2). This interaction was not significant for serum creatine kinase, white blood cell count, neutrophil and lymphocyte counts and neutrophil:lymphocyte ratio. Whilst recognising this interaction, the interaction between curfew treatment x time which signifies the effect of curfew duration was the primary focus in the results.

(i) Liveweight

There was a significant effect of curfew duration on liveweight ($P<0.001$), where there was a 5.2 % and 7.9% reduction following 12 h and 24 h of food and water deprivation, respectively (Table 2, Figure 3). Significant interactions ($P<0.001$) between curfew treatment x replicate and replicate x time were also evident.

(ii) Hydration measures

As the period of curfew increased, the blood and urine became significantly more concentrated as indicated by the increases in total protein ($P<0.05$), haematcrit % ($P<0.001$) in serum, and osmolality ($P<0.001$) and specific gravity ($P<0.001$) in urine (Tables 2-3 and Figure 3). The interactions between curfew treatment x time for serum osmolality and albumin concentration were not significant. The effect of curfew treatment, which represents the mean of the two sampling times, was significant ($P<0.001$) in the case of serum osmolality.

(iii) Creatine kinase (CK)

Serum CK levels significantly increased ($P<0.001$) with increasing curfew duration (Table 1, Figure 4). It is probable that this was associated with the physical exertions of handling, sampling and having equipment fitted rather than the direct effects of the curfew duration *per se*.

(iv) Cortisol

The serum cortisol concentrations increased with curfew treatment ($P<0.05$), but no interaction between curfew treatment x time was observed. In other words, the mean

cortisol concentration between the curfew treatments significantly differed but there was no significant effect of curfew duration on the cortisol response.

Table 1 Least square means ± sem for curfew treatment, replicate and curfew time and significance of the interactions for plasma concentrations of blood urea nitrogen (BUN), total protein (TP), albumin (Alb), creatine kinase (CK), β-hydroxy butyrate (BHB), cortisol (Cort) and osmolality (Osmol)

Main Effects	**BUN** (mg/dL)	**TP** (g/L)	**Alb** (g/L)	**CK*** (U/L)	**BHB** (mmol/L)	**Cort*** (nmol/L)	**Osmol** (mOsmol/L)
Curfew (C)							
0 h	9.40	65.38	31.30	201.27	0.24	68.64	277.80
12 h	11.79	68.56	32.27	282.33	0.18	82.85	282.48
24 h	11.11	69.34	33.15	310.42	0.18	102.88	285.84
sem	0.43	0.73	0.43	-	0.012		1.18
Significance	*P<0.001*	*P<0.001*	*P<0.05*	*P<0.01*	*P<0.01*	*P<0.05*	*P<0.001*
Replicate (R)							
1	10.06	68.32	31.62	242.61	0.19	85.36	280.34
2	11.47	67.20	32.86	279.30	0.21	83.08	283.74
sem	0.35	0.60	0.35	-	0.010		0.97
Significance	*P<0.01*	*ns*	P<0.05	*ns*	*ns*	*ns*	*P<0.05*
Time							
Curfew start	10.23	67.21	32.15	212.33	0.20	86.15	281.79
Curfew end	11.31	68.31	32.36	319.13	0.20	82.30	282.30
sem	0.26	0.44	0.26	-	0.010		0.83
Significance	*P<0.001*	*P<0.001*	*ns*	*P<0.001*	*ns*	*ns*	*ns*
Interactions							
C x R	*P<0.01*	*P<0.05*	*P<0.05*	*ns*	*ns*	*ns*	*ns*
C x Time	*P<0.001*	*P<0.01*	*ns*	*P<0.001*	*ns*	*ns*	*ns*
R x Time	*P<0.01*	*ns*	*ns*	*ns*	*P<0.001*	*ns*	*ns*
C x R x Time	*P<0.001*	*P<0.01*	*P<0.05*	*ns*	*P<0.001*	*ns*	*ns*

*backtransformed least square means shown

Table 2 Least square means ± sem for curfew treatment, replicate and curfew time and significance of the interactions for a subset of blood haematology measures (red cell count – RCC, Haematocrit – HCT, white blood cell count – WCC, neutrophils – NEU, lympocytes – LYM, neutrophils:lymphocytes ratio – NEU:LYM), liveweight, and urine specific gravity (SG) and osmolality

Main Effects	**RCC** ($x10^{12}$/L)	**HCT** (%)	**WCC** ($x10^{9}$/L)	**NEU*** ($x10^{9}$/L)	**LYM*** ($x10^{9}$/L)	**NEU:LYM**	**Liveweight** (kg)	**Urine** SG	**Urine Osmolality*** (mOsmol/L)
Curfew (C)									
0 h	7.68	33.97	7.31	0.98	4.46	0.52	269.21	1.025	740.58
12 h	7.92	35.55	7.22	1.12	3.92	0.60	261.91	1.027	846.57
24 h	8.13	36.77	7.28	1.03	4.28	0.54	258.14	1.033	911.20
sem	0.16	0.61	0.27				2.55	0.0014	
Significance	*ns*	*P<0.01*	*ns*	*ns*	*ns*	*P<0.05*	*P<0.05*	*P<0.001*	*P<0.001*
Replicate (R)									
1	7.82	35.21	7.60	1.10	4.32	0.56	265.32	1.027	826.39
2	8.00	35.65	6.94	0.99	4.18	0.55	260.86	1.029	845.00
sem	0.13	0.50	0.22				2.08	0.011	
Significance	*ns*	*ns*	*P<0.05*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
Time									
Curfew start	7.80	34.93	7.48	1.00	4.56	0.52	268.99	1.024	733.16
Curfew end	8.03	35.93	7.07	1.08	3.89	0.59	257.19	1.033	927.06
sem	0.09	*P<0.001*	0.17				1.48	0.001	
Significance	P<0.001		*P<0.01*	*P<0.05*	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.001*	P<0.001
Interactions	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*P<0.001*	*ns*	P<0.01
C x R	*P<0.001*	*P<0.001*	*P<0.01*	*P<0.05*	*P<0.001*	*P<0.01*	*P<0.001*	*P<0.001*	P<0.001
C x Time	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*P<0.001*	*P<0.001*	P<0.001
R x Time	*P<0.05*	*P<0.01*	*ns*	*ns*	*ns*	*ns*	*P<0.001*	*P<0.001*	P<0.001
C x R x Time									

*backtransformed least square means shown

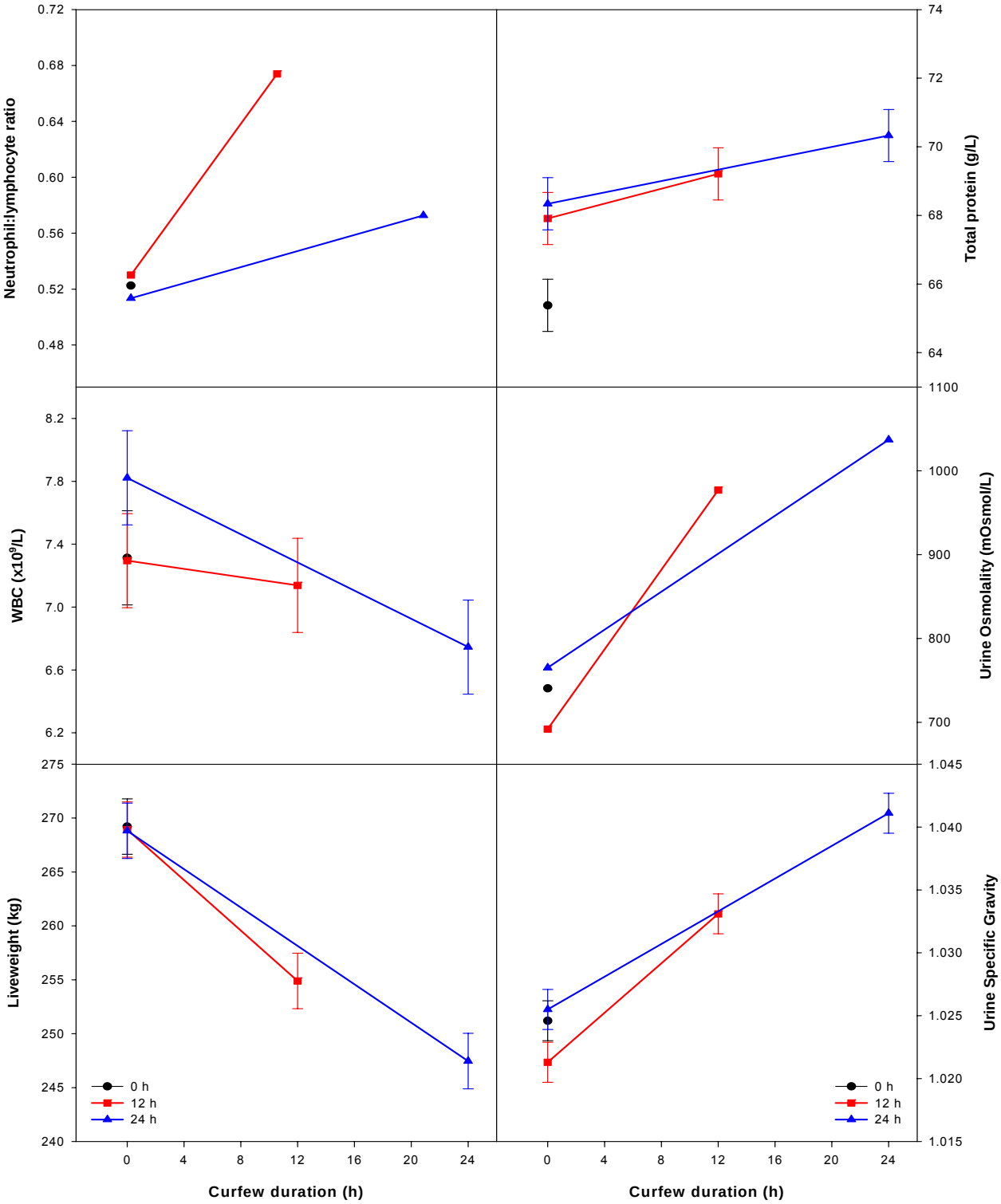

Neutrophil:lymphocyte ratio
0.72
0.68
0.64
0.60
0.56
0.52
0.48
Total protein (g/L)
74
72
70
68
66
64
WBC (x10⁹/L)
8.2
7.8
7.4
7.0
6.6
6.2
Urine Osmolality (mOsmol/L)
1100
1000
900
800
700
Liveweight (kg)
275
270
265
260
255
250
245
240
Urine Specific Gravity
1.045
1.040
1.035
1.030
1.025
1.020
1.015
0 h
12 h
24 h
0
4
8
12
16
20
24
Curfew duration (h)
Curfew duration (h)

Figure 3 Least square means for the effect of curfew duration (interaction curfew x time) on liveweight, urine specific gravity and osmolality, white blood cell count (WBC), neutrophils:lymphocytes ratio and serum total protein.

(v) Haematology
The results for a subset of haematology measures are presented in Table 2 and Figure 3. the curfew periods caused a significant increase and decrease in the red blood cell ($P<0.001$) and white blood cell ($P<0.01$) counts, respectively. Within the white blood cells, the sub-populations of neutrophils ($P<0.05$) and lymphocytes ($P<0.001$) were also significantly influenced by the interaction between curfew treatment x time. However, the changes were not linear with increasing curfew duration. This was illustrated in the trend for neutrophil:lymphocyte ratio ($P<0.001$) which increased over the curfew period but the increase was significantly greater for the 12 h of curfew relative to the 24 h curfew duration (Figure 3).

(vi) Blood urea nitrogen (BUN) and β-hydroxy butyrate (BHB)
For the blood metabolic markers BUN and BHB, the effect of curfew duration was only significant in the case of BUN ($P<0.001$). There was no clear or consistent trend with respect to the changes in BHB. For example, the least square means for the interaction curfew treatment x replicate x time ($P<0.001$) reveal contrasting trends in BHB concentration between replicates for curfew duration (ie curfew treatment x time).

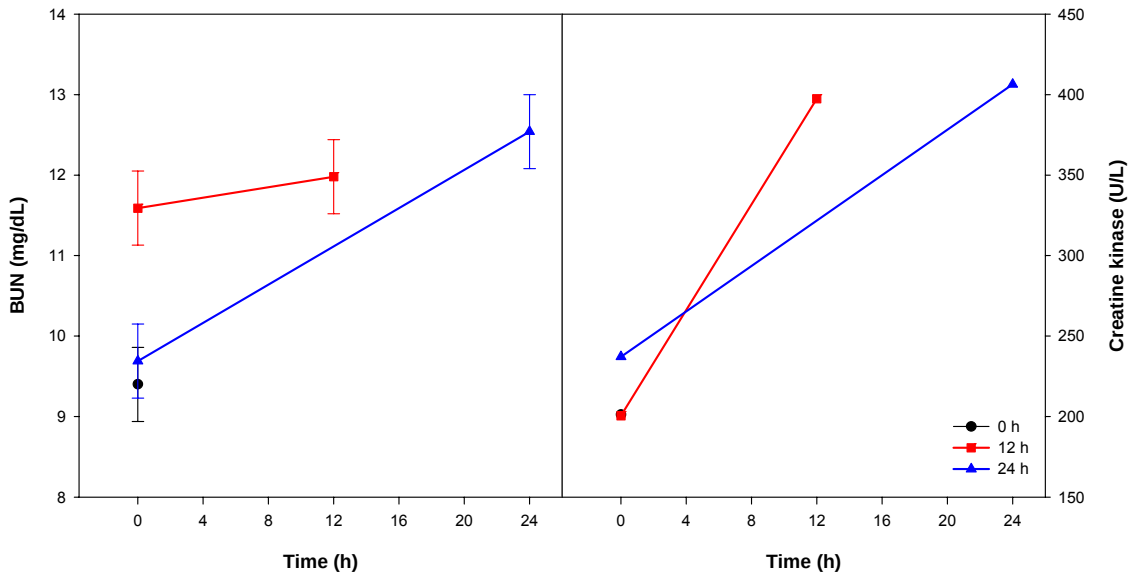


Figure 4 Least square means for the effect of curfew duration (interaction curfew x time) on serum concentrations of blood urea nitrogen (BUN) and creatine kinase (CK)

3.2 Effect of curfew and transport duration on physiological measures during and after transport

A significant four-way interaction between curfew treatment x transport treatment x replicate x time was found for a number of measures (liveweight, urine osmolality and specific gravity, red blood cell count, haematocrit % and the serum concentrations of BUN, total protein and albumin). This interaction generally showed that the key effect of interest, namely the magnitude of the interaction between curfew treatment x transport treatment x time varied between replicates. Variation between replicates was expected. In view of this, and in the interests of simplicity, emphasis was given to the three-way interaction between curfew treatment x transport treatment x time.

(i) Liveweight
There was a significant difference in the pre-transport liveweights as a consequence of the curfew treatments. The significant interaction between curfew treatment x transport treatment x time ($P<0.05$) was largely influenced by the differences observed pre-transport and immediately post-transport. On arrival, the transport mediated losses in liveweight were inversely related to the curfew duration. For example, the liveweight lost over 24 h of transport was 26.6, 18.0 and 13.7 kg for the 0, 12 and 24 h curfew treatments, respectively. The livewieght differences between the different curfew x transport treatments at 24, 48 and 72 h post-transport were not significant. This indicates that although there were significant differences in liveweight on arrival between the treatment groups, this was rapidly attenuated during the 72 h recovery period. At the conclusion of the recovery period, the cattle had returned to 93 – 95 % of their pre-transport mean liveweight.

(ii) Hydration measures
Several of the indicators of hydration status (serum osmolality, urine specific gravity and osmolality, Tables 3 and 4) were significantly influenced by the interaction of curfew treatment x transport treatment x time ($P<0.001$). The temporal changes over the transport and recovery phases were very similar for these measures (Figure 5). As the pre-transport period of food and water deprivation increased, these measures increased, although there were some inconsistencies and in some cases, the differences were not always apparent. The period of transport resulted in further concentration of both blood and urine indicating further dehydration. The combined effects of curfew and transport duration were generally additive, where urine osmolality and specific gravity and serum osmolality increased as the cumulative period of feed and water deprivation increased. In some instances, the differences were not always consistent or large in magnitude. For example, for urine osmolality immediately post-transport, only the 0 h curfew + 12 h transport group significantly differed from the other five treatment groups.

The interaction of curfew treatment x transport treatment x time was not significant for all hydration measures, the notable exceptions being serum albumin and total protein and haematocrit % (Tables 3 and 4). However, although not significant, a similar temporal trend was observed where there was a general increase in these measures (ie. haemoconcentration) as the cumulative period of feed and water deprivation increased. Significant interactions between curfew treatment x time (total protein and haematocrit % $P<0.001$) and transport treatment x time (albumin $P<0.01$) were also observed for these measures.

(iii) Creatine kinase
A significant interaction between curfew treatment x time ($P<0.001$) was found where the CK levels were higher in the 24 h curfew treatment at each of the post-transport time points (Table 3, Figure 7). This was particularly evident immediately on arrival. The interaction between transport treatment x time was also just significant ($P=0.05$) and it showed that transport caused the serum CK levels to increase and that the magnitude was directly related to the transport duration (Table 3, Figure 7). During recovery, the CK concentrations very quickly returned to their pre-transport levels and there was no sustained effect of transport duration. However, there was an indication of a sustained curfew duration effect where the CK levels were significantly higher ($P<0.01$) for the 24 h curfew group relative to the other two treatments, even after 24 h of recovery.

(iv) Cortisol
A significant interaction between curfew treatment x transport treatment ($P<0.05$) was found for serum cortisol concentration. Overall, however, the changes in cortisol over time and indeed in response to the either the curfew or transport treatments do not indicate any consistent or obvious trends.
(v) Haematology
The temporal changes in the overall and differential white blood cell (neutrophils and lymphocytes) counts significantly differed between the curfew treatment x transport treatment groups (Table 4, Figure 5). However, apart from the very obvious transport-mediated increase in white blood cell counts and neutrophil:lymphocyte ratio, there were no clear trends with regard to the interaction between curfew and transport duration.

The changes in red blood cell count over time differed between the curfew treatments ($P<0.001$).

(vi) Blood urea nitrogen (BUN) and β-hydroxy butyrate (BHB)
The interaction curfew treatment x transport treatment x time was significant for BUN ($P<0.001$) and BHB ($P<0.05$) (Table 3, Figure 6). With the exception of the curfew 0 h + transport 12 h group, the serum concentrations of BUN increased in response to transport. The levels on arrival were generally reflective of the total cumulative time of feed and water deprivation. During recovery, the temporal changes were quite variable between the curfew x transport groups. For BHB, the interpretation of the interaction was difficult because of a lack of clear or consistent changes over time between the treatment groups.

Table 3 Least square means ± sem for curfew treatment, transport duration, replicate and time and significance of the interactions for serum concentrations of blood urea nitrogen (BUN), total protein (TP), albumin (Alb), creatine kinase (CK), β-hydroxy butyrate (BHB), cortisol (Cort) and serum osmolality (Osmol)

Main Effects	**BUN** (mg/dL)	**TP** (g/L)	**Alb** (g/L)	**CK*** (U/L)	**BHB** (mmol/L)	**Cort*** (nmol/L)	**Osmol** (mOsmol/L)
Curfew duration (C)							
0 h	12.82	65.66	31.51	336.47	0.21	61.89	281.89
12 h	14.33	68.11	32.31	351.05	0.19	67.31	285.36
24 h	12.78	68.69	32.55	560.95	0.19	76.43	288.09
sem	0.30	0.61	0.40	-	0.007	-	0.70
Significance	*P<0.001*	*P<0.01*	*ns*	*P<0.01*	*P<0.05*	*P=0.07*	*P<0.001*
Transport duration (T)							
12 h	12.96	67.62	32.16	408.27	0.19	64.34	285.55
24 h	13.66	67.36	32.08	401.06	0.20	72.61	284.68
sem	0.24	0.49	0.33	-	0.006	-	0.57
Significance	*P=0.05*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
Replicate (R)							
1	13.14	68.19	31.69	378.15	0.20	69.61	284.00
2	13.47	66.78	32.55	433.01	0.19	67.22	286.23
sem	0.24	0.49	0.33	-	0.006	-	0.57
Significance	*ns*	*P<0.05*	*P=0.07*	*ns*	*ns*	*ns*	*P<0.01*
Time							
Pre-transport	11.31	68.31	32.34	319.13	0.20	82.30	282.30
0 h post-transport	13.77	70.58	34.16	847.06	0.17	55.48	291.02
24 h post-transport	14.53	66.96	31.38	440.19	0.21	78.06	284.80
48 h post-transport	13.70	66.29	31.68	339.15	0.17	72.00	285.78
72 h post-transport	13.23	65.30	31.06	268.83	0.22	56.47	281.66
sem	0.23	0.42	0.28	-	0.008	-	0.81
Significance	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.001*
Interactions							
C x T	*ns*	*ns*	*ns*	*ns*	*P<0.05*	*P<0.05*	*ns*
C x R	*ns*	*P=0.08*	*ns*	*ns*	*P<0.01*	*ns*	*ns*
C x Time	*P<0.001*	*P<0.001*	*ns*	*P<0.001*	*P<0.05*	*ns*	*P<0.001*
T x R	*P<0.001*	*ns*	*ns*	*ns*	*P<0.05*	*P<0.05*	*P<0.01*
T x Time	*P<0.001*	*ns*	*P<0.01*	*P=0.05*	*ns*	*P=0.08*	*P<0.001*
R x Time	*P<0.05*	*ns*	*ns*	*ns*	*P<0.001*	*ns*	*P<0.01*
C x T x R	*ns*	*ns*	*ns*	*ns*	*P<0.01*	*P<0.001*	*P<0.01*
C x T x Time	*P<0.001*	*ns*	*ns*	*ns*	*P<0.05*	*ns*	*P<0.001*
C x R x Time	*P<0.001*	*ns*	*P<0.05*	*P<0.01*	*P<0.05*	*ns*	*P<0.001*
T x R x Time	*P<0.001*	*ns*	*ns*	*ns*	*ns*	*ns*	*P<0.001*
C x T x R x Time	*P<0.01*	*P<0.001*	*P<0.01*	*ns*	*ns*	*ns*	*P<0.01*

*backtransformed least square means shown

Table 4 Least square means ± sem for curfew treatment, transport duration replicate and time and significance of the interactions for a subset of blood haematology measures (red cell count – RCC, haematocrit – HCT, white blood cell count – WCC, neutrophils – NEU, lympocytes – LYM, neutrophils:lymphocytes ratio – NEU:LYM), liveweight, and urine specific gravity (SG) and osmolality.

Main Effects	**RCC** ($x10^{12}$/L)	**HCT** (%)	**WCC** ($x10^{9}$/L)	**NEU*** ($x10^{9}$/L)	**LYM*** ($x10^{9}$/L)	**NEU:LYM**	**Liveweight** (kg)	**Urine SG**	**Urine Osmol*** (mOsmol/L)
Curfew duration (C)	7.80	34.89	7.81	1.25	3.75	0.90	254.76	1.031	914.53
0 h	7.83	35.60	7.91	1.33	3.78	0.95	251.67	1.031	943.08
12 h	8.05	36.94	7.74	1.33	3.56	0.95	247.16	1.035	975.85
24 h									
	0.13	0.47	0.27			0.07	2.37	0.0008	21.70
sem	*ns*	*P<0.05*	*ns*	*ns*	*ns*	*ns*	*P=0.08*	*P<0.001*	*ns*
Significance									
Transport duration (T)	7.82	35.50	7.66	1.28	3.53	1.00	251.91	1.031	914.08
12 h	7.96	36.12	7.98	1.32	3.87	0.87	250.49	1.033	974.89
24 h									
	0.10	0.39	0.22			0.07	1.94	0.0006	17.73
sem	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*P<0.05*
Significance									
Replicate (R)	7.75	35.33	8.13	1.33	3.82	0.94	254.0	1.031	927.46
1	8.03	36.29	7.52	1.27	3.57	0.92	248.4	1.033	961.50
2									
	0.10	*0.39*	0.22			0.07	1.94	0.0006	17.73
sem	ns	*ns*	*P=0.05*	*P<0.05*	*ns*	*ns*	*P<0.05*	*ns*	*ns*
Significance									
Time	8.03	35.93	7.07	1.08	3.89	0.68	*257.19*	1.033	895.13
Pre-transport	8.03	36.22	9.14	2.11	2.96	1.89	*240.44*	1.045	1124.08
0 h post-transport	7.95	36.33	7.55	1.09	4.02	0.64	*252.31*	1.028	887.70
24 h post-transport	7.86	35.97	7.65	1.17	3.87	0.70	*252.43*	1.027	909.92
48 h post-transport	7.59	34.60	7.71	1.27	3.79	0.77	*253.62*	1.027	905.60
72 h post-transport									
	0.08	0.32	0.20			0.07	*1.45*	0.0008	21.50
sem	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	*P<0.001*	*P<0.001*	*P<0.001*
Significance									
Interactions	*ns*	*ns*	*ns*	*P<0.01*	*P=0.07*	*P<0.01*	*ns*	*ns*	*ns*
C x T	*ns*	*ns*	*ns*	*P<0.05*	*P<0.05*	*P<0.05*	*ns*	*ns*	*ns*
C x R	*ns*	*ns*	*P<0.05*	*P<0.001*	*P<0.001*	*P<0.01*	*P<0.001*	*P<0.001*	*P<0.001*
C x Time	*P<0.01*	*P<0.05*	*ns*	*ns*	*P<0.01*	*ns*	*P<0.05*	*P<0.01*	*P<0.05*
T x R							*P<0.001*	*ns*	*P<0.05*
T x Time							*P<0.001*	*P<0.01*	*ns*
R x Time							*P<0.001*	*P<0.05*	*P=0.06*
C x T x R							*P<0.05*	*P<0.001*	*P<0.001*
C x T x Time							*P<0.001*	*P<0.05*	*P<0.01*
C x R x Time							*P<0.001*	*P<0.05*	*ns*
T x R x Time							*P<0.001*	*P<0.001*	*P<0.001*
C x T x R x Time									

*backtransformed least square means shown

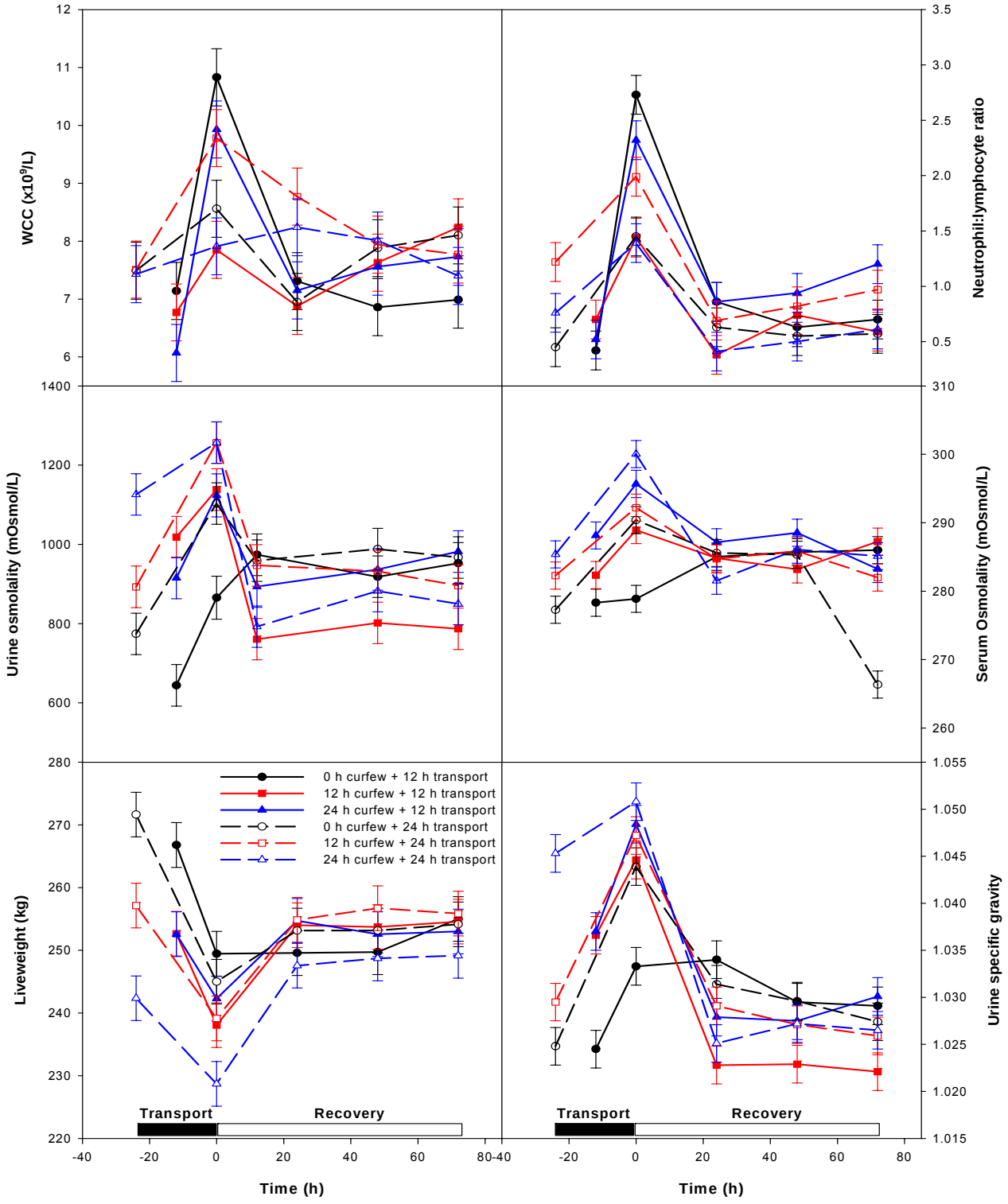


Figure 5 Least square means for liveweight, urine specific gravity and osmolality, serum osmolality, white blood cell count (WCC) and neutrophil:lymphocyte ratio for the interaction between curfew duration x transport duration x time

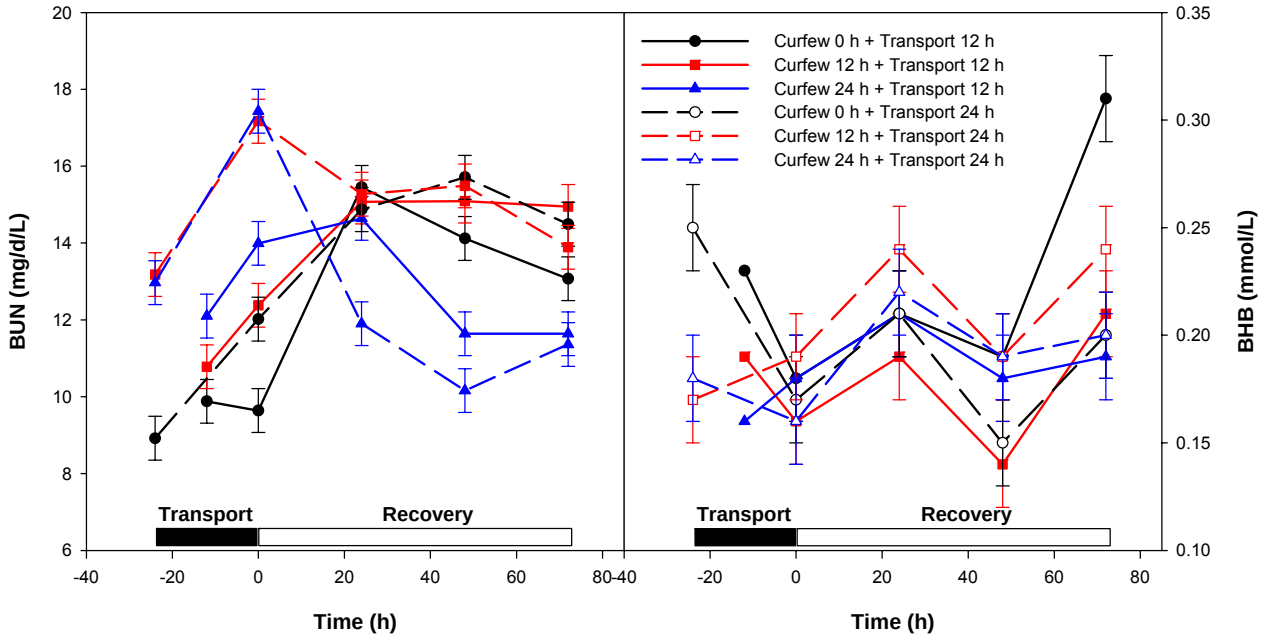


Figure 6 Least square means for blood urea nitrogen (BUN) and β-hydroxy butyrate (BHB) for the interaction between curfew duration x transport duration x time

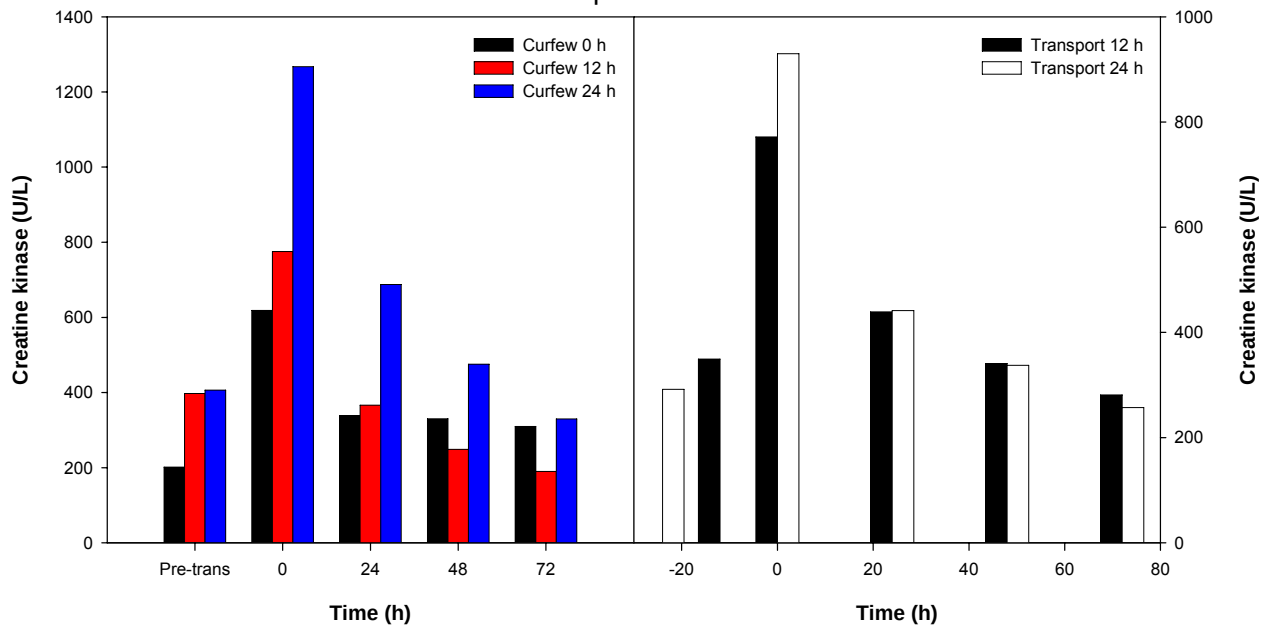


Figure 7 Least square means for serum creatine kinase concentration for the interactions between curfew treatment x time (P<0.001) and transport treatment x time (P=0.05).

3.3 Effect of curfew and transport duration on lying behaviour during and after transport

(i) During the journey

The incidence of lying behaviour during transport is shown in Table 5. Over the shorter duration of 12 h, there were 2 – 3 animals that lay down for short periods. The obvious exception here was animal no. 82 who was lying for a cumulative period of approximately 6 h over the 12 h journey. Clearly, over the longer journey of 24 h, more animals lay down and this typically occurred during the last 8 h of transport. The most telling observation here was that this was only evident for the animals curfewed for 24 h. Moreover, irrespective of transport duration, the incidence of lying behaviour during transport in the cattle that were not curfewed was extremely low. It was only observed in 2 cases during the 12 h transport treatment.

Table 5 Incidence and duration of lying during transport

Animal No.	Curfew Duration (h)	Transport Duration (h)	Lying time during transport (min)
7	0	12	2.4
19	0	12	8.9
37	12	12	11.0
62	12	12	0.6
82	12	12	362.6
20	24	12	5.0
79	24	12	3.0
84	24	12	3.0
3	24	24	29.4
10	24	24	6.0
14	24	24	16.2
24	24	24	767.4
34	24	24	0.6
45	24	24	9.6
66	24	24	40.8
70	24	24	79.2
87	24	24	11.4

(ii) During initial 24 h of recovery

In the repeated measures analysis of lying behaviour during the initial 24 h of recovery, a significant interaction ($P<0.001$) between curfew treatment x transport treatment x

recovery period (initial 24 h) was found (Figure 8). During the initial 3 h of recovery, cattle transported for 24 h lay down significantly longer than those transported 12 h, irrespective of their curfew treatment. During the second 3-h recovery period, there was a pronounced increase in lying time (60 – 70 % time was spent lying), but there were no significant differences between the treatment groups. There were further differences between the six treatment groups at subsequent recovery periods but there was no consistent pattern evident. A bimodal pattern in lying behaviour was evident over the initial 24 h where the peaks occurred during 3 – 6 h and 16 – 18 h post-arrival.

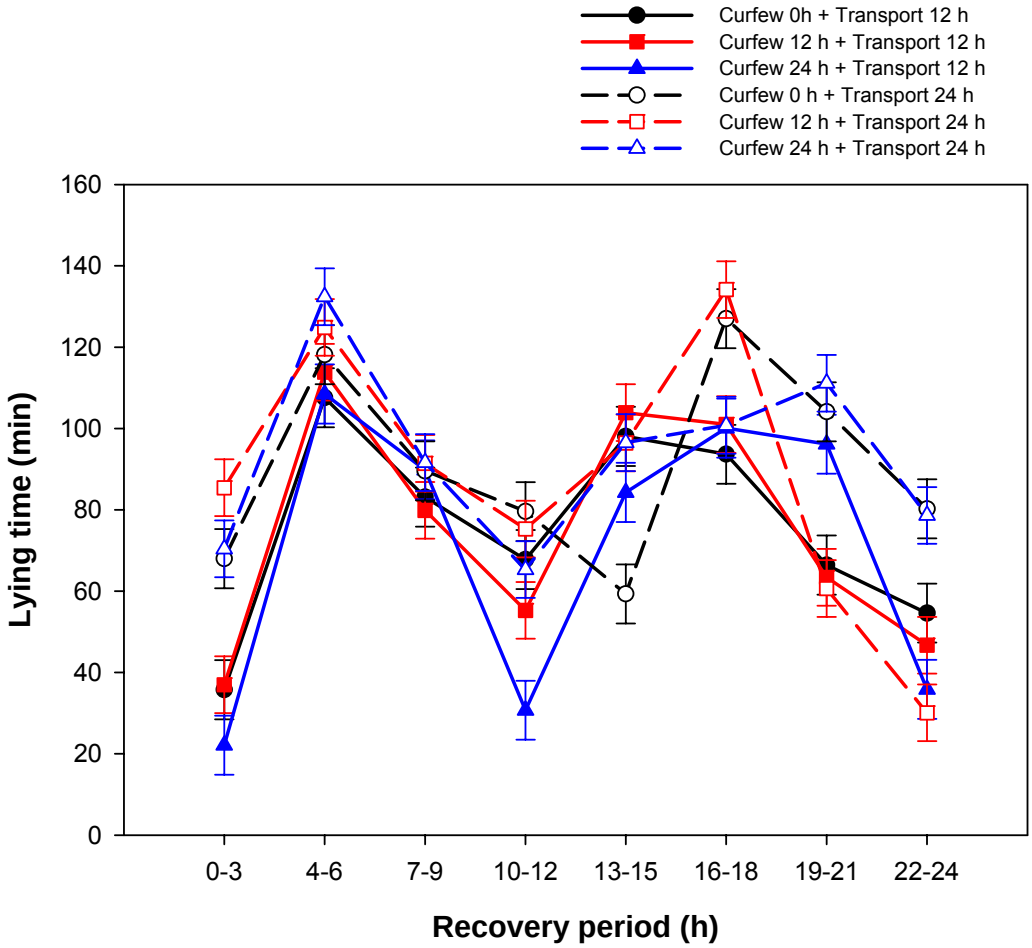


Figure 8 Least square means for lying time over the initial 24 h of recovery for the interaction between curfew duration x transport duration

(iii) During 72 h recovery

Over the full 72 h of recovery, the interaction between curfew treatment x transport treatment x recovery period (3 x 24 h) was significant ($P<0.001$). During the initial 24 h period, there was a clear difference between the transport duration groups and this was once again independent of curfew duration (Figure 9). The pattern between the treatment groups over the next 2 days of recovery was less consistent, but in general, the amount of time lying tended to decline.

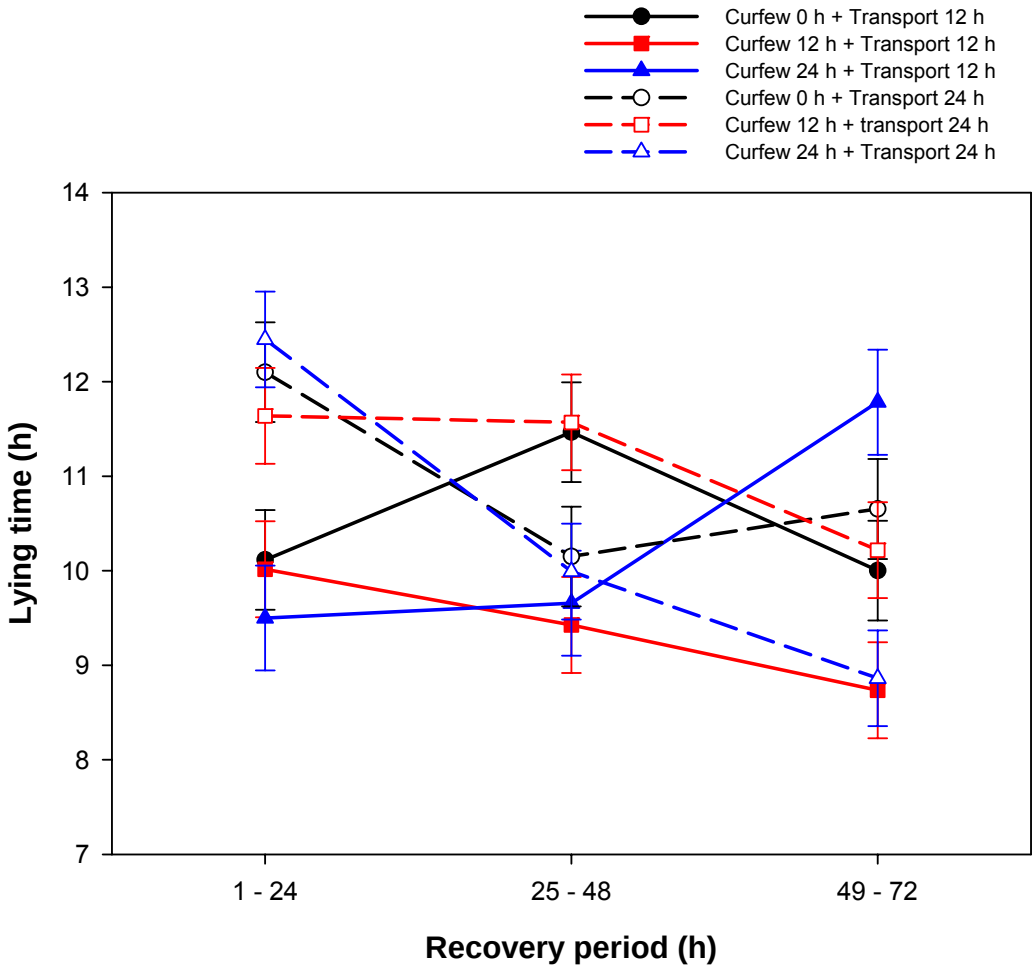


Figure 9 Least square means for lying time over the three 24-h periods of recovery for the interaction between curfew duration x transport duration

3.4 Effect of curfew and transport duration on water consumption during 72 h of post-transport recovery

(i) During initial 24 h of recovery

The consumption of water during the initial 24 h between the six curfew x transport groups in shown in Figure 10. The largest differences between the groups was evident during the initial 3-h recovery period. The volume of water consumed tended to increase as the cumulative period of water deprivation during curfew and transport increased, however the interaction between curfew x transport duration was not significant. Furthermore, the main effects of transport duration and curfew duration just failed to achieve significance. The water consumption during the next 9 h was very consistent between the groups. Some further variability was observed during the remaining periods.

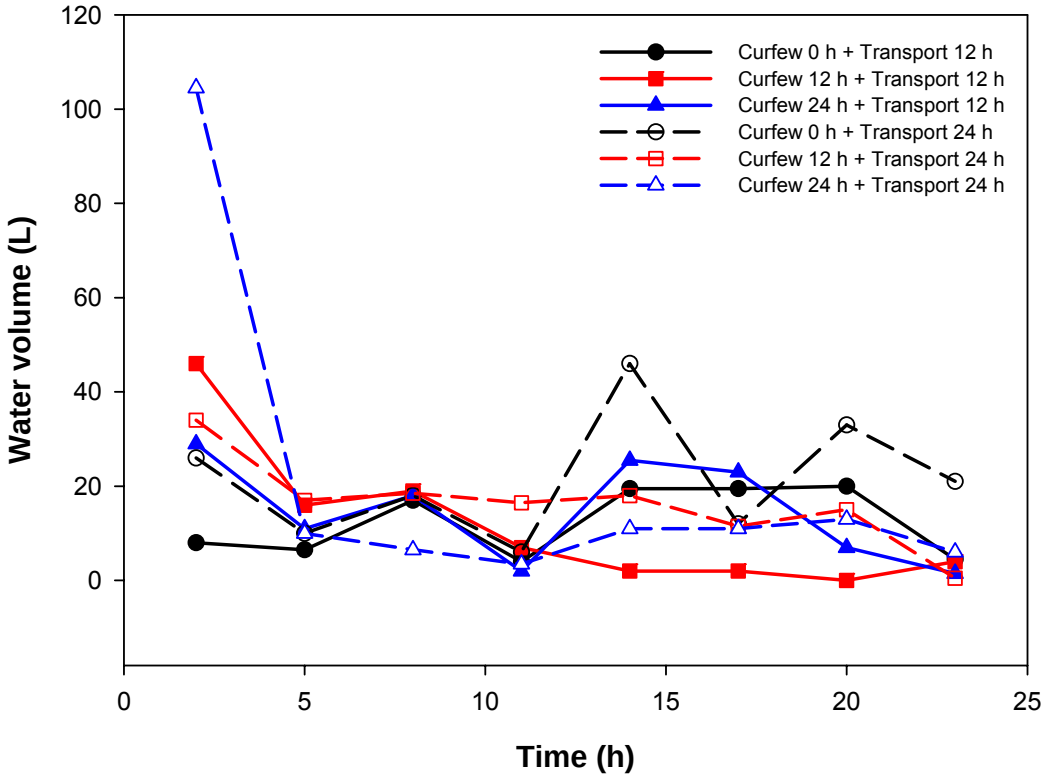


Figure 10 Mean water volume consumed during the initial 24 h of recovery for the different curfew x transport duration treatments

(ii) During 72 h recovery

The 72-h recovery period was divided into 3 periods of 24 h. The results showed a significant effect ($P<0.05$) of period where the cattle drank significantly more during the first 24-h period compared to the second and third periods. Furthermore, there was a significant interaction ($P<0.01$) between transport duration x period (Table 6). Those groups transported for 24 h drank more during the first period and less during the two subsequent 24 h recovery periods.

Table 6 Effect of transport duration on water consumption over the three 24-h periods of recovery (least square means ± sem)

Transport Duration	Recovery Period (h)		
	1 - 24	25 - 48	49 - 72
12 h	107.0	108.3	109.8
24 h	144.3	84.3	90.5

sem	18.9	9.4	7.0

3.5 Specific curfew x transport treatment contrasts

To facilitate further interpretation of the results, two key treatment group contrasts are shown for selected measures in Figures 11 and 12. In Figure 11, the contrast between curfew 12 h + transport 12 h and curfew 0 h + transport 24 h is shown. These were chosen as the total cumulative time off feed and water was the same but occurred under different circumstances. In Figure 12 the contrast between curfew 12 h + transport 24 h and curfew 0 h + transport 24 h is shown primarily to highlight any effects of a curfew prior to a significant transport event.

The changes in the various measures over the transport phase were very similar for the contrast between curfew 12 h + transport 12 h and curfew 0 h + transport 24 h treatments (Figure 11). The curfew 12 h + transport 12 h treatment group tended to lose more weight relative to the other group after transport. During recovery, the profiles were very similar with the exception of urine osmolality which remained higher for the curfew 0 h + transport 24 h group.

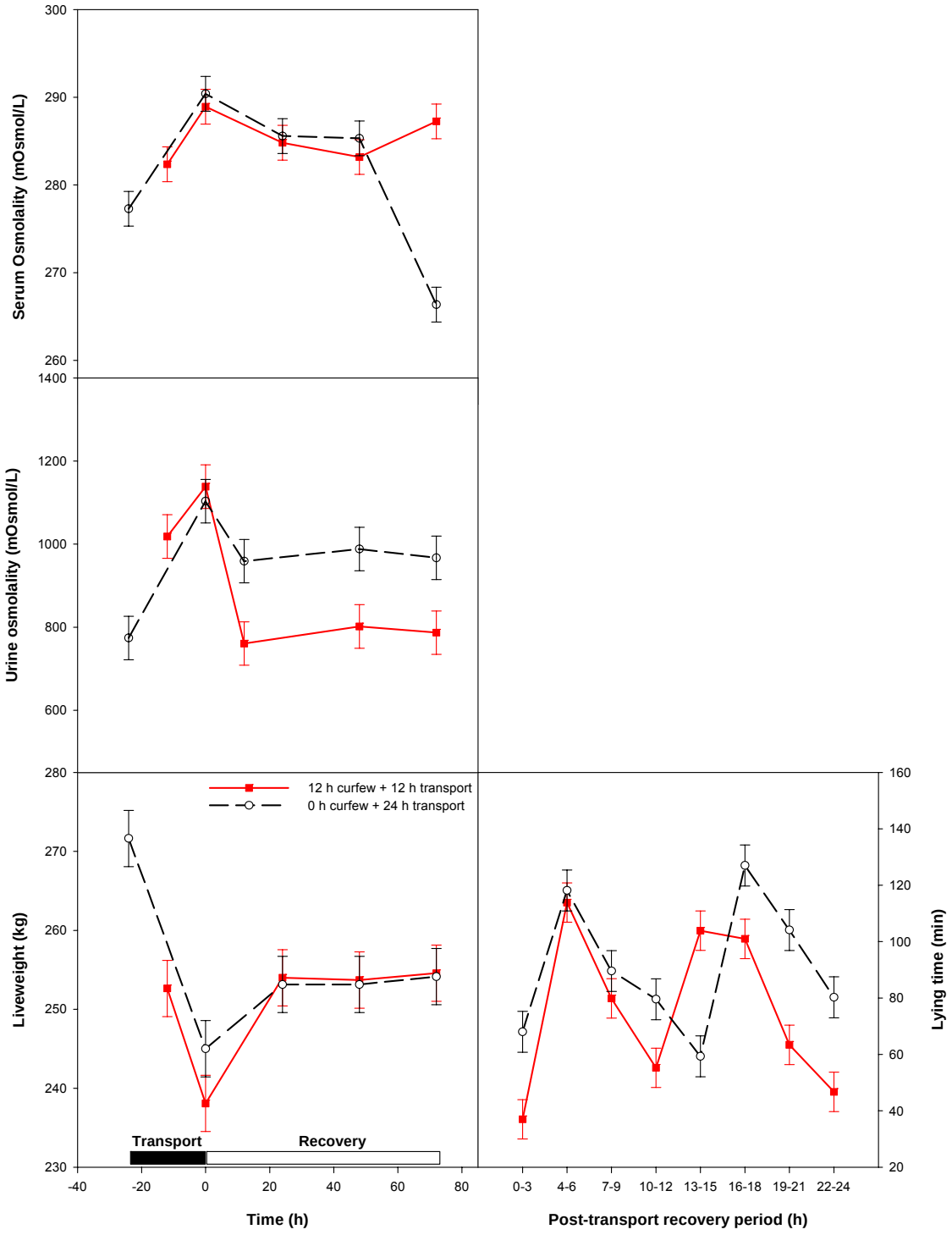


Figure 11 Effect of curfew duration x transport duration on changes in liveweight, urine osmolality and serum osmolality over time and on lying behaviour during the initial 24 h of recovery for the specific contrast between curfew 12 h + transport 12 h versus curfew 0 h + transport 24 h.

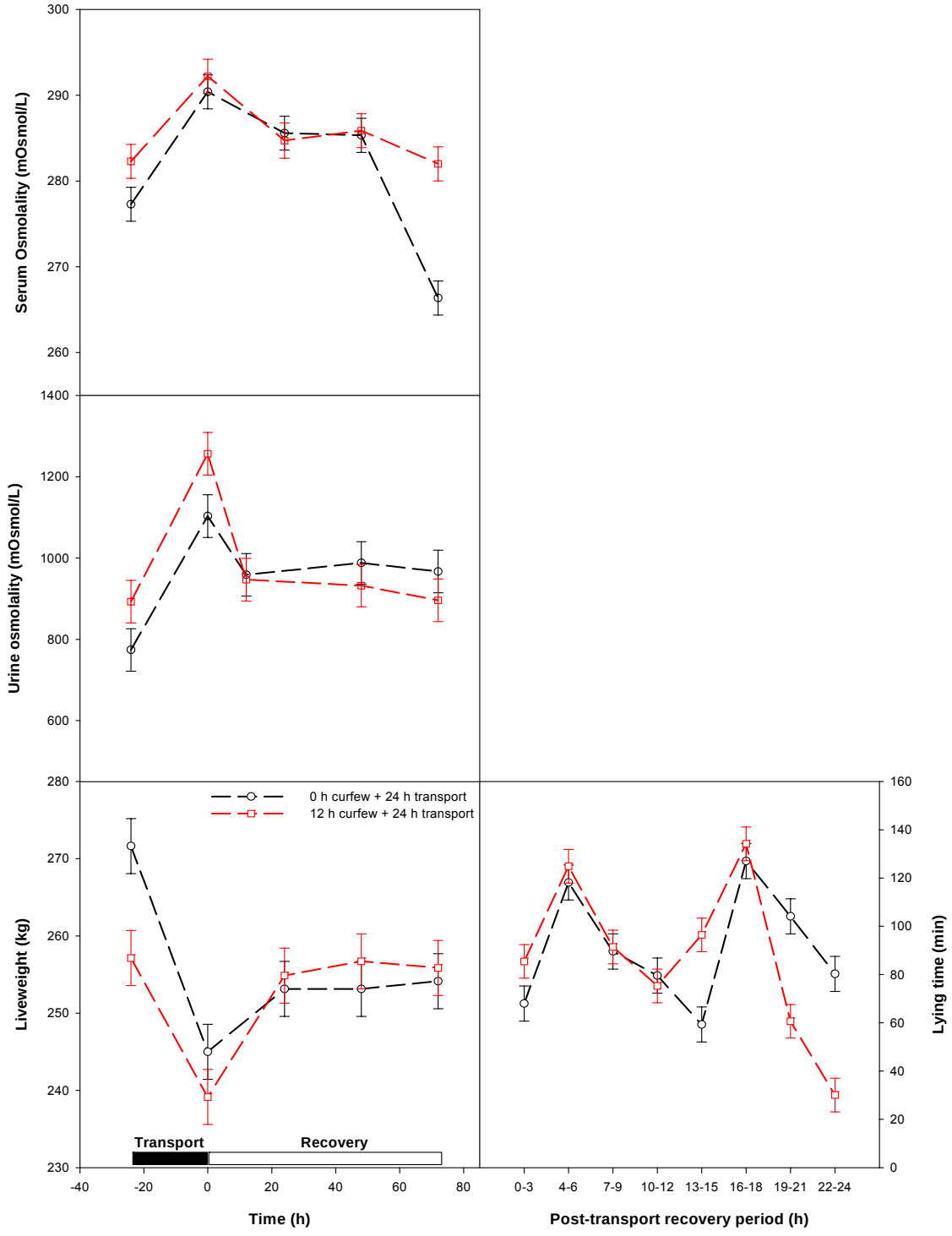


Figure 12 Effect of curfew duration x transport duration on changes in liveweight, urine osmolality and serum osmolality over time and on lying behaviour during the initial 24 h of recovery for the specific contrast between curfew 12 h + transport 24 h versus curfew 0 h + transport 24 h.

The contrast between curfew 12 h + transport 24 h and curfew 0 h + transport 24 h revealed only small differences across the response variables during the transport phase, and these were even less apparent during recovery. Liveweight loss and urine osmolality were higher for curfew 12 h + transport 24 h group after transport and this can be attributed to the increased time of feed and water deprivation.

4. Discussion

The results of this investigation indicate that subjecting healthy, grass-fed cattle to pre-transport periods of food and water deprivation (12 and 24 h compared with 0 h controls) before transport for 12 or 24 h did not enhance the capacity of these animals to cope with transport.

During livestock transport, particularly over moderate to long durations (12 – 48 h), it is the physiological states of fatigue and dehydration that are of most concern from an animal welfare perspective. With regard to dehydration, the results based on the haemoconcentration and urinary measures indicate that the combination of the curfew and transport periods were additive in their effect on hydration status. Put simply, fluid loss, as indicated by these measures, increased commensurate with the total combined period of water deprivation. The water consumed by the treatment groups during the initial 3 h of recovery was also indicative of the total period of water deprivation, particularly at the extremes (i.e. 12 versus 48 h).

There are a small number of investigations comparing the physiological effects of similar periods of food and water deprivation or transport in both cattle and sheep (Gaylean et al 1981, Knowles et al 1995, Parker et al 2003a). The evidence from these studies suggests that the differences in the physiological responses to similar periods of food and water deprivation achieved through a convential curfew or transport are negligible. These results seem counterintuitive given the additional psychological stress and physical demands that occur during transport. Psychological stress can induce diuresis (Parker et al 2003b) and therefore, it is reasonable to expect that fluid losses might be higher during transport. The psychological stress associated with transport, based on changes in heart rate and plasma cortisol concentrations, was generally highest during loading and initial phases of transport (Eldridge et al 1988, Warriss et al 1995, Pettiford et al unpublished). Beyond that, animals generally habituate to the transport conditions. Consequently, the elevated stress response is generally not sustained over the entire journey. The effort to maintain balance during transport would also be expected to incur increased muscular demands compared to that during food and water deprivation only. However, Knowles et al (1995) reported no difference in plasma creatine kinase levels (an indicator of muscle fatigue and use) in sheep transported for 24 h versus those deprived of food and water for 24 h.

The combination of 24 h of curfew followed by 24 h of transport, which represents the maximum time of water deprivation allowable under the welfare transport code, elicited the greatest changes in both urine and haemoconcentration measures. However, these changes were generally within the normal physiological limits. The one exception was urine specific gravity where the maximum normal limit has been reported at 1.045 for cattle (Alexander 1995). Four of the treatment groups had levels at or in excess of this threshold immediately after transport. Whilst useful, some care is required in the

interpretation of measures of urinary concentration such as specific gravity and osmolality, as increased urine concentration is a normal adaptive response to heat stress and/or water deprivation. Whilst high values were seen in the present study, this might not be indicative of clinical dehydration, rather it is probably more reflective of effective renal function. The results for serum osmolality, one of the more accurate measures of haemoconcentration, were similar, albeit slightly lower, to those observed in the previous AHW.055 study where the cattle were transported for 48 h (Pettiford et al 2005).

The behavioural response during and subsequent to transport and serum creatine kinase levels provide insights into the level of fatigue experienced by the cattle. For serum creatine kinase, there were effects of transport duration but the differences were not large between the treatments. The differences in creatine kinase between the curfew treatments, particularly between the 24 h and other two treatments, on arrival and even 24 h post-transport were, inexplicably, much larger. It is worth noting that, although informative, the measure of creatine kinase has limitations with regard to the quantification of muscle fatigue in response to transport. The behavioural responses, specifically lying behaviour, are a much more direct measure and the capability to objectively measure this using the IceTag^{TM} technology greatly added to the study. Unfortunately, it was not possible to detect slippage events using the monitors. The incidence of lying behaviour was more apparent during the longer 24 h journey particularly during the latter stages. This concurs with the results of Knowles et al (1999) where cattle were also transported for 24 h. The most salient feature of the results of the current study was that only those cattle curfewed for 24 h lay down during the 24 h journey. This was not evident for either the 0 or 12 h curfew groups. Moreover, only 2 out of the 17 head that lay down in transit received no curfew. These outcomes are in contrast to the anecdotal views and observations from livestock transporters in relation to non-curfewed cattle during transport, which suggest that non-curfewed cattle are more likely to go down during transit, particularly in the earlier stages of a journey (i.e. before they have had a chance to 'empty out'). The cattle in the present study appeared less likely to lie down if they were not curfewed, and lying events occurred at the end of journeys rather than the beginning.

The proportion of time spent lying during the initial period of recovery was influenced more by the transport duration than any interaction between curfew and transport duration. The results also tend to confirm the earlier observations by Pettiford et al (2005). In their study, cattle transported for 48 h compared to 12 h were more fatigued and spent more time lying during the initial 3 h recovery period. However, this trend was less evident during the second 3 h post-transport period suggesting that cattle transported for 48 h had recovered sufficiently after 3 h. In the current study the transport duration difference in lying behaviour were similarly not evident after the initial 3 h of recovery.

Of the serum measures of metabolic change, BUN was more sensitive to the total period food deprivation than BHB. Inexplicably, the levels of serum BHB declined in response to the curfew and transport treatments. This was also observed by Knowles et al (1999) and Pettiford et al (2005) in cattle transport studies investigating the effect of transport duration. Prolonged fasting has generally been shown to increase lipid catabolism

resulting in higher blood levels of BHB (Blood and Radostits 1989), yet this was not observed in any of three studies where cattle were subjected to food deprivation periods up to 48 h. Knowles et al (1999) attributed the decrease to the combined effects of the journey and the change in diet prior to transport. Certainly, the results suggest that healthy well-fed cattle can tolerate feed deprivation of 48 h without lapsing into a ketotic state.

From the perspective of total time of feed and water deprivation, the changes in liveweight and blood haematology, particularly in relation to the total and differential white cell counts, were similar with those observed by Pettiford et al (2005).

Returning to the original objective of this research which was to establish whether pre-transport curfews facilitate improvements in the capacity of cattle and sheep to cope with transport, there was very little evidence to support this. Rather, the evidence in this study would suggest curfews may be more detrimental based on behavioural data during transport over longer durations. Furthermore, pre-transport curfews (without water in particular) merely extend the total period of time the animals are deprived of water during the transport event. The one caveat with respect to the current study that needs to be recognised is that study was undertaken in June when the pasture conditions were quite dry. The evidence of Gregory et al (2000) indicates that off lush pasture, the level of excreta in the stockcrate will increase substantially in non-curfewed cattle compared with those that were fasted or fed hay. This could exacerbate slippage during the journey and there is also the food safety issue of faecal contamination of the hides. Therefore, there may be some circumstances where a short pre-transport curfew (e.g. 6 h) may be desirable and this is unlikely to be deleterious on animal welfare grounds.

5. Conclusions

The results of this investigation indicate that subjecting healthy, grass-fed cattle to pre-transport periods of food and water deprivation (12 and 24 h compared with 0 h) prior to transport for 12 or 24 h did not enhance the capacity of the animals to cope with transport. The results also indicate that periods of food and water deprivation prior to transport are simply additive to transport-related periods of deprivation, and provide further evidence that healthy cattle under non-threatening environmental conditions can tolerate periods of up to 48 h of feed and water deprivation without undue compromise to their welfare.

6. References

Alexander P (1995). Series A, No 20 Control and therapy of diseases of cattle. University of Sydney Postgraduate Foundation in Veterinary Science, Sydney.

Blood D C, Radostits O M. 1989. Veterinary Medicine, 7th Edn. Baillière Tindall, London.

Eldridge, G.A., Winfield, C.G. and Cahill, D.J. (1988). Responses of cattle to different space allowances, pen sizes and road conditions during transport. *Aust. J. Exper. Agric.* **28**:155-159.

Galyean, ML, Lee, RW and Hubbert, ME (1981). Influence of fasting and transit on ruminal and blood metabolites in beef steers. *J. Anim. Sci*. **53**: 7-18.

Gregory, NG, Jacobsen, LH, Nagle, TA, Muirhead, RW and Leroux, GJ (2000). Effect of preslaughter feeding system on weight loss, gut bacteria and the physico-chemical properties of digesta in cattle. *NZ J. Agric. Res.* **43**: 351-361.

Jones, GE and Mould, DL (1984). Adaptation of the guaiacol (peroxidase) test for haptoglobins in a microtitration plate system. *Res. Vet. Sci*. **37**:87-92.

Kaneoko, JJ, Harvey, JW and Bruss, M.L. Eds. (1997). "Clinical Biochemistry of Domestic Animals" 5th Edition. (Academic Press, London, UK).

Knowles, TG, Warriss, P D, Brown, S N & Edwards, J E 1999. Effects on Cattle of Transportation by Road for up to 31 Hours. *Vet. Rec*. **145**: 575-582.

Knowles, TG, Brown, SN, Warriss, PD, Phillips, AJ, Dolan, SK, Hunt, P, Ford, JE, Edwards, JE and Watkins, PE. (1995). Effects on sheep of transport by road for up to 24h. *Vet. Rec*. **136**: 421-438.

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

Parker, AJ, Hamlin, GP, Coleman, CJ and Fitzpatrick, LA (2003b). Dehydration in stress ruminants may be the result of cortisol-induced diuresis. J. Anim. Sci. 81: 512-519.

Parker, A.J., Hamlin, G.P., Coleman, C.J. and Fitzpatrick, L.A. (2004). Excess cortisol interferes with a principal mechanism of resistance to dehydration in *Bos indicus* steers. *J. Anim. Sci*. **82**: 1037-1045

Pettiford, S.G., Ferguson, D.M., Lea, J.M., Lee, C., Paull, D., Reed, M.T. and Fisher, A.D. (2006). Milestone Report - The effect of transport duration on indicators of cattle welfare. MLA Project AHW.055 – Animal welfare outcomes of livestock road transport practices.

Slocombe, L.L. and Colditz, I.G. (2005). Evaluating the stress of production in cattle using haptoglobin. 5th Annual International Colloquium on Animal Acute Phase Proteins, Dublin, Ireland.

Warriss, P.D., Brown, S.N., Knowles, T.G., Kestiin, S.C., Edwards, J.E., Dolan, S.K. and Phillips, A.J. (1995). Effects on cattle of transport by road for up to 15 hours. *The Vet. Rec.* **126**:319-323.

Overall progress of the project

The project continues to track well and is on schedule. The next experiment examining the effect of transport duration on the behavioural and physiological responses in mature sheep is due to commence on 30 October 2006.

Recommendations

The general practice of pre-transport curfews for cattle is highly variable in its application in terms of the duration of curfew and whether the cattle are deprived of feed or water or both. The generic application of some period of pre-transport curfew, irrespective of the transport duration and nutritional background of the cattle, is highly questionable in terms of benefits. The outcomes of this investigation indicate that subjecting healthy, grass-fed cattle to pre-transport periods of food and water deprivation (12 and 24 h compared with 0 h) prior to transport for 12 or 24 h did not enhance the capacity of the animals to cope with transport. Given this, it is recommended that the need for pre-transport curfews should be predicated on consideration of key factors such as the nutritional background and condition of the cattle and the duration of the transport.

Milestone 8

Report on the completion of the stage 1 experiment (sheep) detailing results and recommendations of the most informative welfare indicators for further validation in stage 2.

Abstract

The aim of this experiment was to quantify the impact of transport duration on behavioral and physiological indicators of sheep welfare. One hundred and twenty mature Merino ewes (mean liveweight 46.6 ± 4.6 kg) were used for the experiment. The design comprised 3 transport duration treatments of 12, 30 and 48 h and these were replicated over 2 weeks. The sheep were transported using single trailer commercial trucks. The departure times were staggered to allow the trucks to arrive on the same day. On arrival, the sheep were allocated to pens with access to hay and water. Detailed measurements including liveweight, blood chemistry and haematology and urine specific gravity were collected on 20 focal animals on each vehicle pre-transport, immediately on arrival and 24, 48 and 72 h after arrival. Vaginal temperature was logged over the entire transport and 72 h post-transport phases. Variation was observed between the transport treatment groups and the replicates for some of the pre-transport measures. On arrival, a significant 3-way interaction between transport duration x replicate x sampling time (post-transport) was observed for urine specific gravity, nearly all the haematology and several of the blood chemistry measures. Where this wasn't the case, the interaction between transport duration x sampling time (post-transport) was significant for all measures with the exception of serum cortisol and monocyte count. The largest variation between the transport duration treatments was observed immediately on arrival. Sheep transported over longer durations (30 or 48 h) were more dehydrated on arrival and consumed more water during the initial stages of recovery. However, the levels of haemoconcentration were not sufficient to be of clinical concern. There was a positive trend between transport duration and liveweight loss. The sheep regained liveweight during the initial 72 h of post-transport recovery (91 – 96 % of their pre-transport liveweight) and it took between 7 – 14 days to recover or exceed the liveweight lost through transport. There was consistent difference in lying behaviour post-transport. The results of this study generally indicate that healthy sheep that have not had restricted access to food or water prior to transport can tolerate transport up to 48 h without any major compromise to their welfare.

Project objectives

1. Determine the contribution of handling, loading and initial transport processes on the stress responses of cattle to road transport.
2. Determine the animal welfare outcomes of yearling cattle transported under controlled conditions for 6, 12, 30 or 48 hours from farm to feedlot entry.
3. Determine the animal welfare outcomes of mature sheep (eg. live export trade) transported under controlled conditions for 12, 30 or 48 hours.
4. Utilize a readily-applicable subset of welfare measures to determine the typical welfare outcomes of yearling cattle and cast for age sheep transported under a range of commercial conditions.
5. Develop recommendations on best practice yearling cattle and cast for age sheep transport management for optimal welfare and productivity.

Success in achieving milestone

THE EFFECT OF TRANSPORT DURATION ON INDICATORS OF SHEEP WELFARE

Drewe Ferguson, Dom Niemeyer, Caroline Lee, David Paull, Jim Lea, Matt Reed and Andrew Fisher

1. Introduction

The standards of husbandry and welfare practiced during livestock production are becoming important factors influencing consumer perceptions in many markets. Clearly, the use of welfare-unfriendly livestock transport practices has the potential to downgrade product quality. Furthermore, the use of practices that initiate market and public concerns which can't be adequately addressed may cause damage to the image and market access of Australian livestock products.

Unfortunately, the ability of Australian livestock industries to demonstrate the welfare status of livestock during land transport remains vulnerable on several grounds. There is little scientific evidence that the welfare of cattle and sheep trucked under Australian conditions is superior or equivalent to that of animals transported by our competitors and international trading partners. Further, there are some marked differences in our conditions and practices compared with those of others. If Australian practices and regulations are going to be different of necessity from those of other trading blocs, then we may need objective evidence that the animal welfare outcomes are equivalent and acceptable. Consequently this project was designed to develop scientifically defensible quantification of the animal welfare outcomes of Australian livestock transport practices. The research reported here represents part of a series of investigations examining the animal welfare outcomes of land transport practices for cattle and sheep.

In Australia, sheep can be transported over considerable distances and durations. Under the Australian Model Code of Practice for the Land Transportation of Sheep (1983), the maximum allowable duration is primarily determined by the maximum time that sheep can be deprived of water. For adult sheep, the maximum duration is 36 h with the option to extend to 48 h if the animals are not displaying obvious signs of fatigue, thirst or distress and if the extension allows the journey to be completed within 48 h. These times have subsequently been revised in the draft code which has not yet been ratified but includes the maximum allowable water deprivation time of 32 h but this can be extended to 38 h if the journey can be completed within that time.

The Australian evidence supporting the establishment of the original and revised maximum periods is very limited and indirect. Given the paucity of direct evidence, this investigation was undertaken to specifically examine the impact of transport duration on sheep welfare indicators.

2. Materials and Methods

The experiment was approved by the CSIRO Livestock Industries FD McMaster Laboratories Animal Ethics Committee (Approval No. 06/43).

2.1 Sheep

A total of 1610 sheep was used in the experiment. All the sheep were derived from research and station flocks located at the FD McMaster Laboratories Chiswick field station. Of these, a group of 120 Merino ewes were used as focal animals where detailed measurements were recorded. The ewes were between 4 – 5 years of age, not pregnant and had approximately 2 months of wool growth (Figure 1). The justification for the use of mature dry Merino ewes as focal animals was based on the industry trends that it is the older cast-for-age animals that are typically transported over longer durations in Australia. The remainder comprised both Merino and Merino x Romney sheep of mixed sex (ewes and wethers) and ages that were used as filler animals on the transport vehicles. This was necessary because it was important that each vehicle carried a near full load in order to achieve the normal conditions (density, movement, etc.) that prevail in commercial stock vehicles.

Figure 1 Focal ewes used in the experiment

2.2 Experimental design and procedures

The experimental design comprised 2 replicates x 3 transport duration treatments of 12, 30 and 48 h and these were conducted over 2 weeks during the month of November. Each week comprised a single replicate.

Two weeks prior to the commencement of the transport phase, the 120 focal animals were weighed and allocated to their transport treatments (n = 20/transport duration x replicate). After weighing, the focal animals from each transport duration treatment were mixed with their respective filler groups to enable the focal animals to adjust to the new social conditions prior to transport. The liveweight range of the filler groups was also measured at this time. All sheep were then maintained on improved pastures prior to their transport treatments. The pasture conditions were reasonably dry (estimated at 50% DM) at the time of the experiment due to below average spring rainfall.

Four hours prior to transport, each transport group was brought into the yards and the focal animals were drafted off from the filler group. The filler animals were returned to a nearby holding paddock with feed (pasture and hay) and water available. The focal animals were weighed and fitted with vaginal temperature loggers and a subset (n=8 - 9) were also fitted with IceTag behavioural loggers. Two blood samples (6ml and 9 ml vacutainers) were taken by jugular venepuncture. Finally, a urine sample (1-3 ml) was collected via a urinary catheter.

Just prior to loading, the focal animals were rejoined with their filler group and loaded on the truck. Very similar commercial single trailer trucks with Byrne stockcrates (4 decks and 4 pens/deck) were used to transport the sheep (Figure 1). Each vehicle was fitted with a GPS and temperature and humidity loggers were secured in the front pens of the two middle decks of the trailer (Tiny Tag plus TGP1500 loggers, Omni Instruments, Scotland - temperature range -30 to +50oC and relative humidity 0 to 100%).

Figure 2 Commercial livestock vehicle used in the experiment

Each truck was loaded with approximately 350 – 450 animals including the 20 focal animals. The pens were loaded according to the recommended stocking density based on liveweight under the model code of practice for the land transport of sheep. The focal animals were allocated to specific pens within the vehicle to ensure that they experienced the range of challenges associated with the journey. The departure of the three vehicles within replicate was staggered so that all arrived on the same day. The sheep were transported over a circuit of 5.5 h comprising both highway and secondary roads. During transport, the sheep were monitored every 2-3 h by the driver according to normal industry practice.

At the conclusion of the journey, the sheep were unloaded and the focal animals were drafted off. The focal animals were weighed and sampled for blood and urine immediately upon arrival and 24, 48 and 72 h after arrival. During the 72 h post-

transport recovery phase, the focal animals were held in yards with access to water and high quality lucerne hay. These yards and pens were familiar to the animals after the 72 h recovery phase, the loggers were removed and the animals were transferred to a pastured paddock. The animals were then reweighed 7 and 14 days after transport.

2.3 Blood sampling and biochemical measurements

Each of the focal animals was blood sampled on 5 occasions (pre-transport, arrival and 24, 48 and 72 h post-transport). At each sampling, 6ml (EDTA vacutainers) and 9 ml (serum separator vacutainers) samples were collected. An aliquot of whole blood was analysed for haematology variables (haemoglobin (HGB), red cell counts (RCC), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelet count (PC), white blood cells count (WBC) and differential cell counts of neutrophils (NEU), lymphocytes (LYM), monocytes (MON), eosonophils (EOS), basophils (BAS)) using a Cell-Dyn Haematology Analyser 3500R (Abbott Diagnostics, CA, USA).

An aliquot of blood was centrifuged and the resultant plasma was harvested and frozen (-20^{o}C) for subsequent analysis of blood chemistry. The concentrations of cortisol, blood urea nitrogen, total protein, albumin, creatine kinase, β-hydroxy butyrate and haptoglobin were determined on the plasma samples. Cortisol was determined using a Spectria Cortisol RIA (Orion Dianostica, Espoo, FIN). An OLYMPUS AU400 automated clinical autoanalyser was used to analyse the blood urea nitrogen, creatine kinase and total protein plasma concentrations. Osmolality was measured, using a vapour pressure osmometer (Wescor, 5500). The acute phase protein haptoglobin was determined in plasma by a variation of the method of Jones and Mould (1984). The assay was modified to account for the effect of free haemoglobin due to haemolysis (Slocombe and Colditz, 2005).

2.4 Urine sampling and measurement

Urine was collected on 5 occasions (pre-transport, arrival and 24, 48 and 72 h post-transport) via insertion of a urinary catheter into the bladder. A small amount of Xylocaine gel was placed on the tip of the catheter to minimise discomfort upon insertion in the urethra. A 5 ml syringe was placed on the end of the catheter and this was used to withdraw 1 - 3 ml of urine from the bladder. The urine was transferred to sterile 5 ml containers.

Urine specific gravity was measured with a veterinary refractometer (DLC Australia Pty Ltd, Caboolture, Qld, 4510 AUS).

2.5 Body temperature

On the day of transport, focal sheep were fitted with vaginal temperature loggers. The temperature loggers (Thermochron iButton, Maxim Integrated Products, USA) were secured to blank (non-progesterone) sheep CIDRs that were placed in the vagina. Body temperature was recorded every 2-3 min until the loggers were removed at 72 h post-transport.

2.5 Behavioural assessments

IceTag™ behavioural monitors (IceRobotics, Midlothian, Scotland, UK) were placed in a canvas sock and this was secured to the right foreleg of each animal with velcro and duct tape. These monitors recorded whether the animal was lying, standing or active (walking, moving) and measurements were recorded every second. Behaviour was monitored during the transport and 72 h post-transport recovery phases.

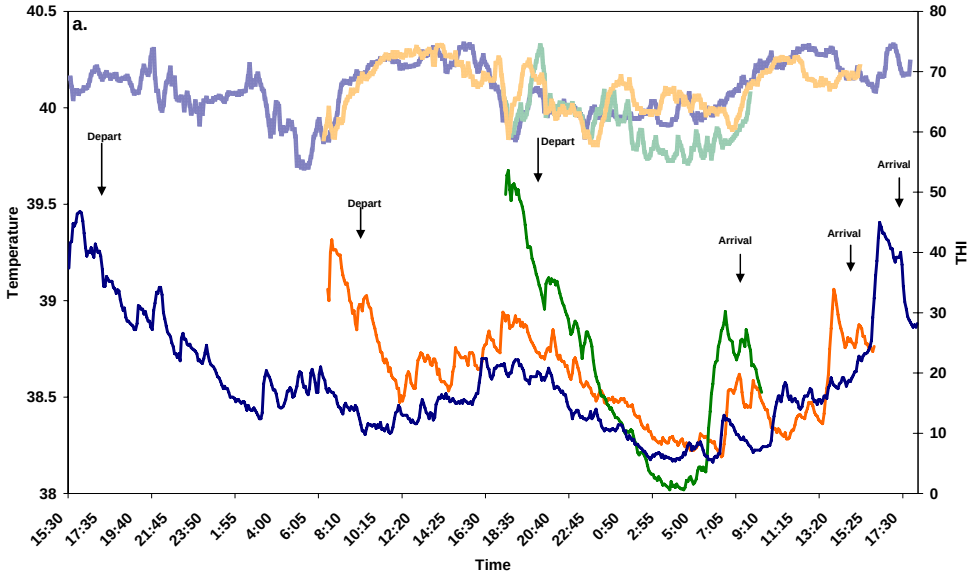
2.6 Statistical analysis

Several blood parameters were transformed to stabilise variances prior to the analysis. The pre-transport measurements were analysed using the GLM procedure in SAS (*SAS* 1999). The initial model contained the fixed effects of transport duration, replicate and their interaction. The post-transport measurements were analysed using the Mixed model procedure in SAS. The initial model contained the fixed effects of transport duration, replicate and sampling time post-transport (Time) and their interactions plus a random term for animal. Non-significant interactions were sequentially removed to reveal the final model.

3. Results

The results of body temperature and on board temperature humidity index (THI) recordings are presented in Figures 3ab.

3.1 Body temperature



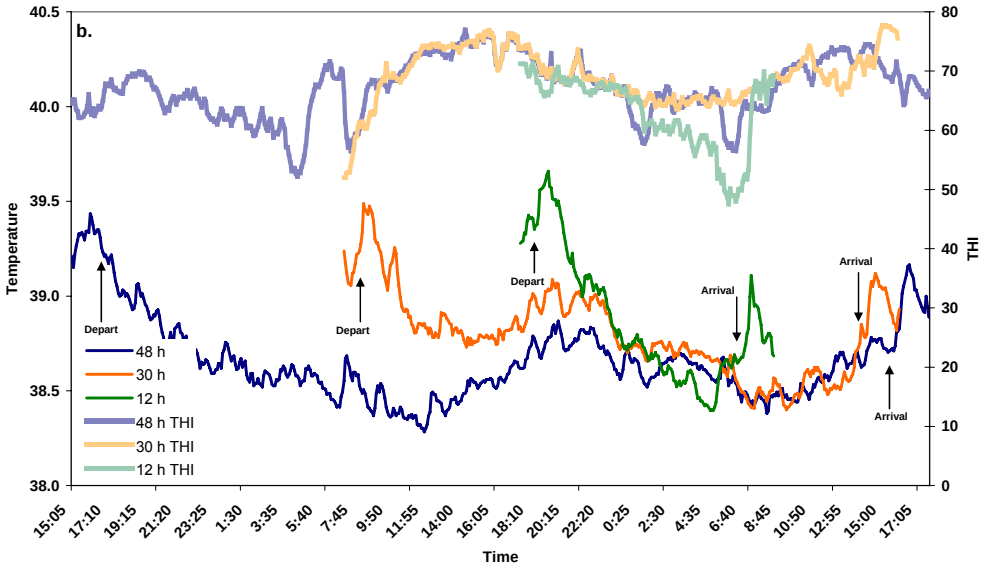


Figure 3ab Mean rectal temperature profiles and on-board THI for the three journey durations during (a) replicate 1 and (b) replicate 2.

The familiar trends of increased body temperature during the process of loading and the initial stages of transport, followed by a decline to more basal temperatures were apparent in this experiment. These results suggest that the sheep habituated to the transport conditions. The combination of unloading and handling at the conclusion of the journey also resulted in an elevation in temperature. However, the temperature increase was generally lower than that observed at the commencement of the journey. For the longer journey durations of 30 and 48 h, a diurnal temperature pattern was evident. This pattern was in concert with changes in THI on-board the truck. The THI data indicates that the on-board conditions were generally comfortable for the animals (THI $\leq$ 70) during the majority of the journey. However, there were short periods (mid afternoon) where the THI increased to levels above 70 and the animals may have experienced some mild heat stress although this was not reflected in body temperature during these periods.

The mean minimum and maximum temperatures and relative humidity during the experimental period in Armidale were 8.1 – 21.3^{o}C and 73.5% (at 9 am). It needs to be remembered that the actual climatic conditions prevailing during the respective journeys will vary slightly from those measured in Armidale.

3.2 Pre-transport measurement of blood chemistry, haematology and urine SG

The pre-transport means for the majority of the blood chemistry and haematology variables (Table 1) generally conformed to the accepted normal clinical ranges for sheep (eg. Kaneko et al 1997). There were exceptions here but it needs to be reinforced that these normal values and ranges are only indicative. The pre-transport cortisol and CK levels were above the normal levels and this was probably attributable to the handling

and movement that occurred prior to sampling. The lower than expected BUN and BHB levels are not cause for concern from a metabolic perspective.

Table 1 Pre-transport means and normal clinical means or ranges for blood parameters

Blood Parameter	Mean	Normal Range or Mean ± sd*
Blood urea nitrogen (BUN mg/dL)	7.05	8.0 – 20.0
Total protein (TP g/L)	67.20	60.0 – 79.0
Albumin (Alb. g/L)	29.42	26.7 – 36.8
Creatine kinase (CK U/L)	174.62	7.7 – 101.0
β-hydroxy butyrate (BHB mmol/L)	0.26	0.55 ± 0.04
Cortisol (nmol/L)	84.0	62.0 ± 10.0
Osmolality (Osmol. mOsmol/L)	298.35	282-297
Haemoglobin (HGB g/L)	146.21	90.0 – 140.0
Red cell count (RCC x 10^{12}/L)	11.68	8.0 – 16.0
Haematocrit (HCT %)	0.35	0.24 – 0.50
Mean corpuscular vol. (MCV fL)	33.61	23.0 – 48.0
Mean conc. HGB in red cells (MCHC g/L)	336.00	310.0 – 380.0
Mean corpuscular HGB (MCH %)	11.28	34.0 – 38.0
Platelets count (PC x 10^9/L)	383.86	250.0 – 750.0
White blood cell count (WCC x 10^9/L)	4.91	4.0 – 12.0
Neutrophils (NEU x 10^9/L)	2.22	0.7 – 6.0
Lymphocytes (LYM x 10^9/L)	1.70	2.0 – 9.0
Mononcytes (MON x 10^9/L)	0.55	0 – 0.75
Eosinophils (EOS x 10^9/L)	0.23	0 – 1.0
Basophils (BAS x 10^9/L)	0.06	0 – 0.4

Differences in pre-transport blood chemistry and haematology measures between transport duration treatments and replicates are presented in Tables 2 and 3, respectively. Significant pre-transport differences between the transport duration x replicate groups were evident for several measures including BUN, TP, ALB, CK, Cort, MCHC, WBC, NEU and LYM. There were also significant pre-transport differences between the transport duration and/or replicate groups for some of the other measures (Osmol, Urine SG, HGB, RCC, HCT and BAS). However, whilst significant, the differences were relatively small in magnitude.

Table 2 Adjusted LS means for pre-transport serum concentrations of blood urea nitrogen (BUN), total protein (TP), albumin (Alb), creatine kinase (CK), β-hydroxy butyrate (BHB), cortisol (Cort) and osmolality (Osmol) and urine specific gravity (SG)

Main Effects	**BUN** (mg/dL)	**TP** (g/L)	**Alb** (g/L)	**CK*** (U/L)	**BHB*** (mmol/L)	**Cort*** (nmol/L)	**Osmol** (mOsmol/L)	**Urine SG**
Duration (D)								
12 h	7.27	65.80	29.12^{a}	175.19	0.26	71.19	297.15^{a}	1.034^{a}
30 h	7.29	66.32	28.56^{a}	110.72	0.27	71.47	296.58^{a}	1.038^{a}
48 h	6.58	69.49	30.59^{b}	138.83	0.23	71.13	291.65^{b}	1.016^{b}
sem	0.15	0.65	0.29	0.002	0.01	0.09	1.91	0.002
Significance	*P<0.01*	*P<0.001*	*P<0.001*	*P<0.001*	*ns*	*ns*	*P<0.001*	*P<0.001*
Replicate (R)								
1	7.16	67.06	29.69	140.27	0.26	63.40	295.03	1.036
2	6.93	67.35	29.15	136.06	0.24	80.11	301.68	1.024
sem	0.12	0.53	0.24	0.002	0.01	0.08	1.56	0.002
Significance	*ns*	*ns*	*ns*	*ns*	*ns*	*P<0.05*	*P<0.01*	*P<0.001*
D x R								
12 h x replicate 1	6.96^{a}	63.64^{a}		136.74^{b}		58.54^{a}		
30 h x replicate 1	7.88^{b}	67.43^{bc}		114.97^{bc}		53.57^{a}		
48 h x replicate 1	6.64^{a}	70.11^{d}		179.08^{a}		81.26^{a}		
12 h x replicate 2	7.58^{b}	67.96^{cd}		230.43^{a}		86.58^{b}		
30 h x replicate 2	6.71^{a}	65.22^{ab}		106.70^{c}		95.35^{bc}		
48 h x replicate 2	6.51^{a}	68.87^{cd}		110.22^{bc}		62.27^{ab}		
sem	0.22	0.92		0.003		0.13		
Significance	*P<0.01*	*P<0.01*	*ns*	*P<0.001*	*ns*	*P<0.01*	*ns*	*ns*

*Backtransformed least square means shown. SEMs are not backtransformed. Means with different superscripts are significantly different P<0.05.

Table 3 Adjusted LS means for pre-transport haematology parameters

Main Effects	HGB	RCC	HCT*	MCV	MCH	MCHC	WBC*	NEU*	LYM*	NEU:LYM	MONO*	EOS*	BAS*
Duration (D)													
12 h	112.96[a]	10.06[a]	0.33[a]	33.08	11.23	339.95	4.85	2.11	1.82	1.24	0.48	0.33	0.049[a]
30 h	120.78[b]	10.74[b]	0.36[b]	33.66	11.25	334.53	4.49	1.89	1.54	1.28	0.46	0.41	0.069[a]
48 h	116.52[a]	10.27[a]	0.35[c]	34.09	11.36	333.43	4.88	2.16	1.72	1.30	0.52	0.35	0.059[ab]
sem	1.43	0.12	0.27	0.31	0.09	1.53	0.04	0.06	0.08	0.07	0.11	0.03	0.005
Significance	*P<0.001*	*P<0.001*	*P<0.001*	*ns*	*ns*	*P<0.01*	*ns*	*ns*	*P=0.06*	*ns*	*ns*	*ns*	*P<0.05*
Replicate (R)													
1	118.51	10.53	0.36	33.73	11.27	334.47	4.74	1.98	1.78	1.17	0.52	0.36	0.055
2	114.99	10.19	0.34	33.49	11.29	337.47	4.75	2.13	1.61	1.38	0.46	0.36	0.063
sem	1.17	0.10	0.22	0.25	0.07	1.25	0.03	0.05	0.07	0.06	0.06	0.02	0.004
Significance	*P<0.05*	*P<0.05*	*P<0.05*	*ns*	*ns*	*ns*	*ns*	*ns*	*P=0.08*	*ns*	*ns*	*ns*	*ns*
D x R													
12 h x wk 1						332.72[bc]	5.35[b]	2.28[ab]	2.13[a]				
30 h x wk 1						339.17[a]	4.45[a]	1.87[a]	1.58[b]				
48 h x wk 1						331.52[b]	4.47[a]	1.81[a]	1.63[b]				
12 h x wk 2						347.18[c]	4.40[a]	1.96[a]	1.51[b]				
30 h x wk 2						329.90[ab]	4.55[ac]	1.93[a]	1.51[b]				
48 h x wk 2						335.34[c]	5.34[bc]	2.57[b]	1.82[ab]				
sem						2.17	0.06	0.08	0.11				
Significance	*ns*	*ns*	*ns*	*ns*	*ns*	*P<0.001*	*P<0.01*	*P<0.05*	*P<0.01*	*ns*	*ns*	*ns*	*ns*

*Backtransformed least square means shown. SEMs are not backtransformed. Means with different superscripts are significantly different P<0.05.

Table 4 Effect of transport duration, replicate and time post-transport on haematology parameters

Main Effects	HGB	RCC	HCT*	MCV	MCH	MCHC	WBC*	NEU*	LYM*	NEU:LYM	MONO*	EOS*	BAS*
Duration (D)													
12 h	114.78	10.33	0.34	32.76	11.11	339.54	5.72	2.77	1.89	1.83	0.53	0.32	0.065
30 h	117.88	10.48	0.35	33.12	11.25	340.26	5.62	2.64	1.83	1.71	0.53	0.35	0.070
48 h	119.13	10.48	0.35	33.50	11.38	339.89	5.39	2.46	1.79	1.64	0.56	0.37	0.062
sem	1.46	0.12	0.27	0.29	0.08	1.37	0.03	0.03	0.07	0.11	0.07	0.02	0.004
Significance	*ns*	*ns*	*P=0.09*	*ns*	*P=0.07*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
Replicate (R)													
1	119.78	10.63	0.36	33.38	11.27	337.87	5.47	2.57	1.83	1.68	0.55	0.33	0.060
2	114.74	10.22	0.34	32.86	11.22	341.91	5.68	2.66	1.84	1.78	0.54	0.36	0.071
sem	1.19	0.12	0.22	0.24	0.07	1.11	0.02	0.03	0.06	0.09	0.05	0.02	0.003
Significance	*P<0.01*	*P<0.01*	*P<0.001*	*ns*	*ns*	*P<0.05*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*P<0.05*
Time post-transport (T)													
0 h	116.92	10.34		33.10	11.32	342.19	6.23	3.66	1.62	2.66	0.59	0.13	0.060
24 h	117.47	10.43	0.34	33.22	11.26	339.28	5.95	2.67	2.02	1.50	0.55	0.52	0.061
48 h	116.97	10.46	0.35	33.02	11.19	339.00	5.19	2.27	1.89	1.40	0.50	0.40	0.058
72 h	117.71	10.49	0.35	33.15	11.23	339.12	5.03	3.90	1.80	1.34	0.53	0.42	0.084
sem	0.09	0.07	0.35	0.17	0.05	0.8	0.02	0.03	0.05	0.09	0.04	0.02	0.003
Significance	*ns*	*P<0.01*	0.17	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.001*
Interactions			*P<0.001*										
D x R	*P<0.05*	*ns*											
D x T	*P<0.05*	*P<0.05*		*ns*	*ns*	*P<0.05*	*P<0.05*	*ns*	*P<0.01*	*ns*	*ns*	*ns*	*ns*
R x T	*P<0.001*	*P<0.001*	*P<0.05*	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.05*	*ns*	*ns*	*ns*	*ns*	*P<0.001*	*P<0.05*
D x R xT	*P<0.001*	*P<0.001*	*P=0.05*	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.01*	*P<0.001*	*P<0.05*	*P<0.001*	*ns*	*P<0.01*	*ns*
			P<0.001	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.01*	*P<0.001*	*P<0.05*	*P<0.05*	*ns*	*P<0.001*	*ns*

*Backtransformed least square means shown. SEMs are not backtransformed

3.3 Effect of transport duration on physiological measures

The significance of the main effects of transport duration, replicate and time (post-transport) and their interactions on the changes in the various physiological measures are shown in Tables 4 and 5.

Significant three-way interactions between transport duration x replicate x time (post-transport) were observed in all but two of the haematology parameters (Table 4) and for several blood chemistry measures and urine SG (Table 5). This interaction generally showed that the key effect of interest, namely the magnitude of the interaction between transport duration x time post-transport varied between replicates. Variation between replicates was expected. In view of this, and in the interests of simplicity, emphasis was given to the two-way interaction between transport duration x time.

Table 5 Effect of transport duration, replicate and time post-transport on liveweight (LWT), serum concentrations of blood urea nitrogen (BUN), total protein (TP), albumin (Alb), creatine kinase (CK), β-hydroxy butyrate (BHB), cortisol (Cort) and osmolality (Osmol) and urine specific gravity (SG)

Main Effects	**LWT** (kg)	**BUN** (mg/dL)	**TP** (g/L)	**Alb** (g/L)	**CK*** (U/L)	**BHB*** (mmol/L)	**Cort*** (nmol/L)	**Osmol** (mOsmol/L)	**Urine SG**
Duration (D)									
12 h	44.42	7.95	66.63	29.61	95.48	0.26	60.48[a]	294.31	1.038
30 h	43.50	8.51	69.78	30.25	115.97	0.30	42.69[b]	294.63	1.047
48 h	42.75	7.28	70.23	30.97	133.47	0.32	44.40[b]	295.77	1.045
sem	0.61	0.18	0.57	0.24	0.002	0.01	0.07	0.75	0.001
Significance	*ns*	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.01*	*ns*	*P<0.001*
Replicate (R)									
1	43.89	8.14	68.53	30.68	113.44	0.26	47.24	294.57	1.044
2	42.42	7.69	69.23	29.88	113.45	0.32	50.0	295.24	1.043
sem	0.50	0.15	0.47	0.20	0.001	0.01	0.06	0.61	0.001
Significance	*ns*	*P<0.05*	*ns*	*P<0.01*	*ns*	*P<0.001*	*ns*	*ns*	*ns*
Time post-transport (T)									
0 h	42.42	7.77	71.73	32.24	168.99	0.34	35.34	298.26	1.052
24 h	43.89	7.33	67.06	29.72	115.18	0.28	71.15	293.26	1.046
48 h	44.21	8.14	68.48	29.51	92.97	0.25	48.81	294.54	1.039
72 h	43.7	8.41	68.25	29.63	95.85	0.30	45.38	293.56	1.037
sem	0.36	0.12	0.38	0.16	0.001	0.001	0.07	0.78	0.001
Significance	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.001*	P<0.001	*P<0.001*
Interactions									
D x R	*ns*	*P<0.001*	*P<0.01*	*ns*	*ns*	*ns*	*ns*	*ns*	*P<0.01*
D x T	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.01*	*P<0.01*	*P<0.001*	*ns*	*P<0.001*	*P<0.001*
R x T	*ns*	*P<0.001*	*P<0.001*	*ns*	*ns*	*P<0.05*	*P<0.05*	*P<0.001*	*P<0.05*
D x R xT	*ns*	*P<0.05*	*P<0.05*	*ns*	*ns*	*ns*	*ns*	*P<0.001*	*P<0.01*

*Backtransformed least square means shown. SEMs are not backtransformed. Means with different superscripts are significantly different P<0.05.

(i) Liveweight

A significant interaction between transport duration x time was found for liveweight (Table 5 and Figure 4a). This was due to the differences between the treatments on arrival where the liveweight loss of the 12 h group was significantly lower than either the 30 or 48 h treatment groups. The differences over the remainder of the recovery were not significant. The magnitude of the liveweight lost through transport was 4.9 %, 9.8 % and 12.1 % for the 12 h, 30 h and 48 h transport duration treatments, respectively. After 72 h of recovery, the treatments groups had recovered to 91 – 96% of the pre-transport liveweight.

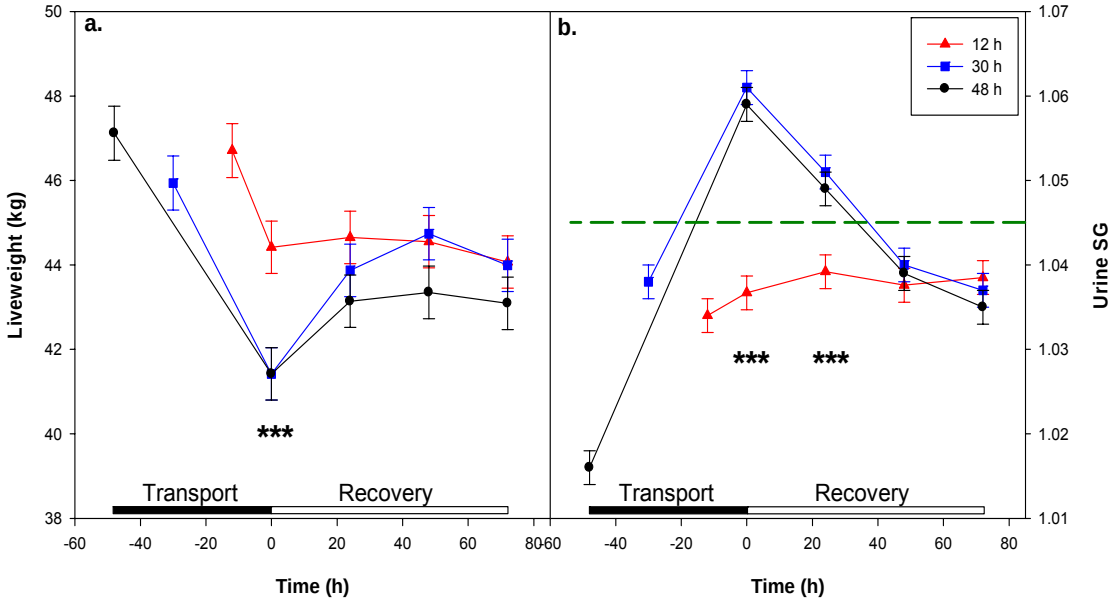


Figure 4ab Changes in (a) liveweight and (b) urine specific gravity (LS means) following 12, 30 or 48 h of transport and during 72 h of post-transport recovery. (* P<0.05, ** P<0.01 and *** P<0.001)

(ii) Haematology

The interaction between transport duration x time was significant for the majority of the haematology measures (Table 4). This interaction was not significant for some of the specific leucocyte populations including neutrophils, lymphocytes and monocytes. Furthermore, these cell populations were not significantly influenced by transport duration but significant differences over the 72 h post-transport recovery period were apparent (Table 4). The post-transport changes in leucocyte numbers (WBC) and the ratio of neutrophyils:lymphocytes (NEU:LYM) are illustrated in Figures 5c and d, respectively. Transport caused pronounced increases in WBC and NEU:LYM but these declined during the post-transport recovery phase to levels similar to that observed prior to transport.

Red blood cell count was also significantly influenced by the interaction of transport duration x time (Table 4, Figure 5a). This was largely due to transport duration treatment differences between sampling time points but not within time points. For the 12 and 48 h

transport groups, RCC tended to increase with transport and during the initial 24 h of recovery before stabilising over the remainder of the post-transport recovery period. The opposite trend was observed for the 30 h treatment group where it declined from the pre-transport levels and increased during the 24 – 72 h of recovery.

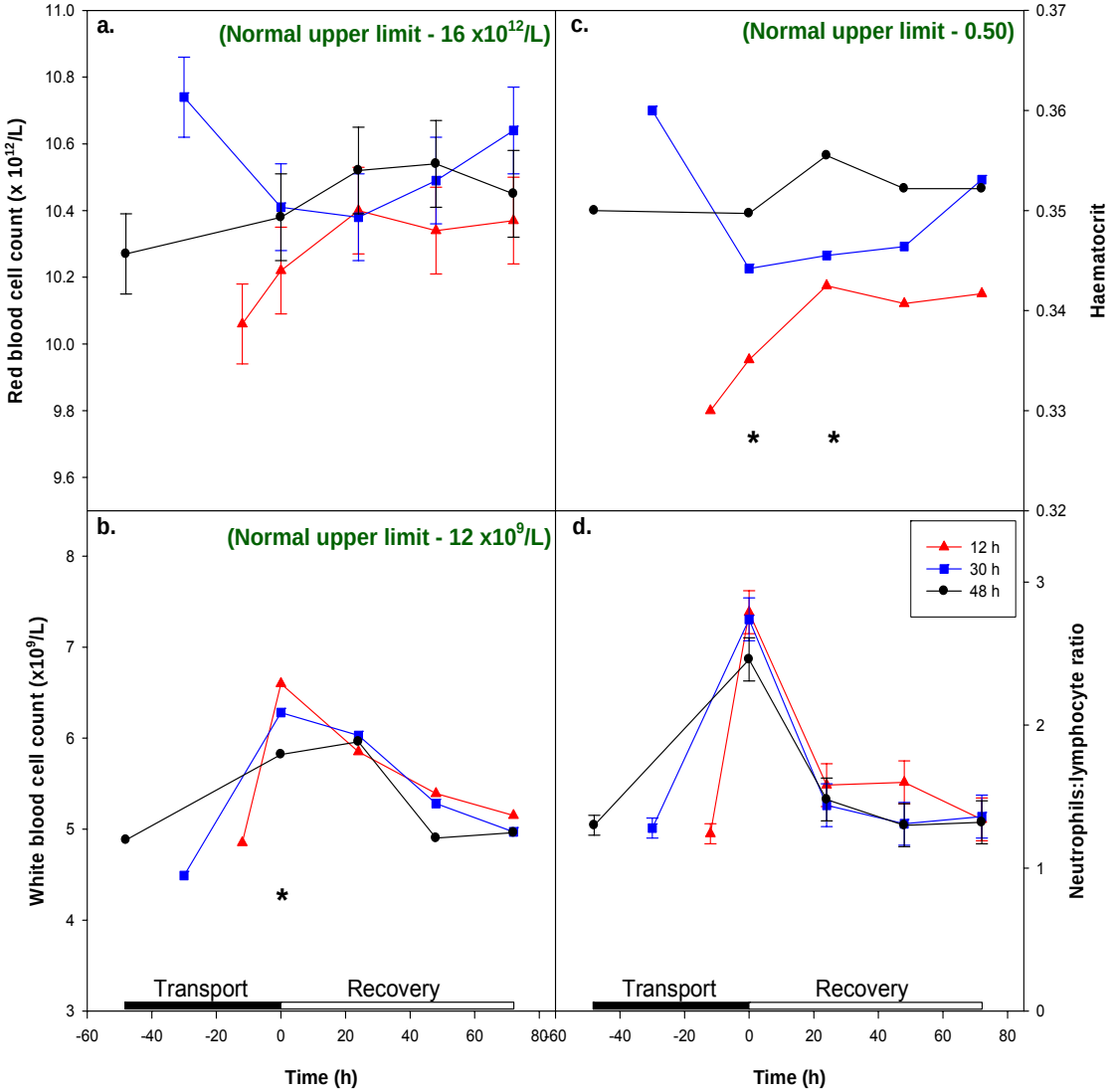


Figure 5abcd Changes in (a) red blood cell count, (b) white blood cell count, (c) haematocrit and (d) neutrophil:lymphocyte ratio (LS means) following 12, 30 or 48 h of transport and 72 h of post-transport recovery. (* P<0.05, ** P<0.01 and *** P<0.001)

The same interaction only just attained significance (P=0.05) for HCT (Table 4) and this was attributable to the 12 and 48 h treatment group means differences at 0 and 24 h post-transport. The trends over time do not show a consistent pattern between the transport duration groups (Figure 5b).

(iii) Blood urea nitrogen (BUN) and β-hydroxy butyrate (BHB)
The measures of serum BUN and BHB concentrations provide an indication of altered metabolic states particularly with regard to protein and lipid catabolism, respectively. Significant differences in both metabolic measures were observed between the duration treatments at the various post-transport sampling times (Table 5 and Figures 6ab) Serum BUN levels generally increased during the transport and recovery phases (Figure 5a). The levels after 72 h of recovery for each transport duration treatment were significantly higher than the levels immediately after transport. Significant differences between the treatment duration groups at each sampling time point were evident but there was no obvious trend with regard to transport duration. BUN levels for the 48 h transport duration group were significantly lower than the levels for the 12 h and/or 30 h groups.

Serum BHB concentration increased as a consequence of transport, mainly for the longer durations of 30 and 48 h, then declined during the initial 48 h of recovery before increasing over the remaining 24 h of recovery (Figure 6b). Significant differences in BHB between the duration treatments were evident on arrival (12 h < 30 and 48 h) and after 24 h of recovery (12 h < 48 h; 30 h < 48 h) but dissipated over the remainder of the recovery phase.

(iv) Creatine kinase (CK)
Transport resulted in an increase in the serum levels of the muscle enzyme CK, with the exception of the 12 h duration group where a decrease was observed (Figure 6c). Serum CK concentration provides an indication of muscle activity and fatigue. CK levels declined over the recovery phase to levels at or below those evident prior to transport. There tended to be a positive trend between transport duration and CK levels (ie 12 h< 30 h < 48 h) which was apparent over the post-transport recovery phase.

(v) Hydration measures
All of the measures that indicate hydration status (haematocrit, serum total protein (TP) and albumin (ALB) concentration, osmolality (Osmol) and urine specific gravity) were significantly affected by the interaction transport duration x time (Table 5). For the serum measures of ALB, TP and Osmol, the temporal changes due to and subsequent to transport were very similar (Figures 6def). The period of water deprivation enforced through transport resulted in haemoconcentration as indicated by the increase in these specific measures. The transport-mediated increase in TP and ALB was dependent on transport duration. The trend was less apparent for serum osmolality where the 12 h treatment mean was significantly lower than the 30 h and 48 h treatment means ($P<0.01$) but the 30 h mean was significantly higher than the 48 h treatment mean ($P<0.05$). The levels for all measures generally declined particularly during the initial 24 h of recovery and after 72 h post-transport, the serum levels were very close to those observed pre-transport. The trends were very similar for urine SG (Figure 4b) although there was very little change over the entire transport and recovery phase for the 12 h treatment group.

(vi) Cortisol
Significant transport duration ($P<0.01$) and time ($P<0.001$) effects were found for serum cortisol concentration (Table 5). A significant interaction between replicate x time was

also observed. The changes in cortisol over time and indeed in response to the transport duration treatments do not indicate any consistent or obvious trends.

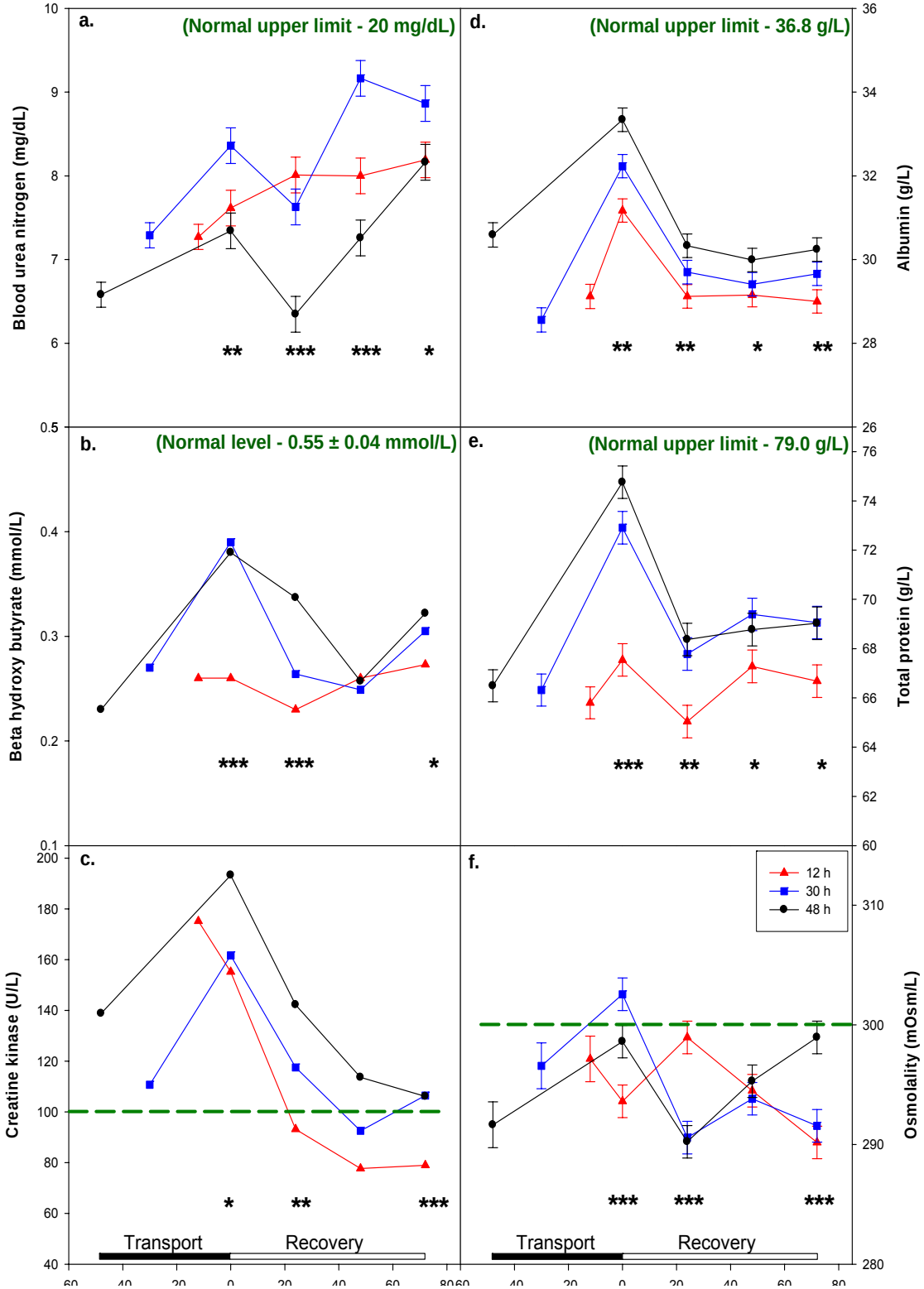

a.
(Normal upper limit - 20 mg/dL)
Blood urea nitrogen (mg/dL)
d.
(Normal upper limit - 36.8 g/L)
Albumin (g/L)
b.
(Normal level - 0.55 ± 0.04 mmol/L)
Beta hydroxy butyrate (mmol/L)
e.
(Normal upper limit - 79.0 g/L)
Total protein (g/L)
c.
Creatine kinase (U/L)
f.
12 h
30 h
48 h
Osmolality (mOsm/L)
Transport
Recovery
Transport
Recovery

Figure 6abcdef Changes in serum (a) blood urea nitrogen, (b) β-hydroxy butyrate (c) cretaine kinase, (d) albumin, (e) total protein and (f) osmolality (LS means) following 12, 30 and 48 h of transport and 72 h of post-transport recovery. (* $P<0.05$, ** $P<0.01$ and *** $P<0.001$)

3.4 Effect of transport duration on lying behaviour

(i) During transport

Of the focal animals fitted with the IceTag behavioural monitors, a total of 2, 5 and 11 animals were found to have spent some time lying (> 1 min) during the 12 h (Mean 44.8 min; Range 25 – 64 min), 30 h (Mean 13.7 min; Range 2 – 39 min) and 48 h (Mean 33.3 min; Range 2 – 80 min) of transport, respectively.

(ii) Post-transport

The initial 24 h of recovery was divided into eight 3-h periods. The time spent lying during the initial 24 h post-transport recovery period, was significantly influenced by the interaction transport duration x replicate x period ($P<0.01$). The simpler interaction between duration x period was also highly significant ($P<0.001$). The changes in the duration treatment LSMs over the 8 periods is shown in Figure 7a. It can be seen that overall, sheep spent very little time lying and recovering from transport. Sheep transported over the longer durations of 30 or 48 h were lying significantly longer than the 12 h treatment group during the periods between 7 – 15 h. In the subsequent period the opposite trend was observed where the 12 h treatment group were lying significantly longer than the 30 h and/or 48 h groups.

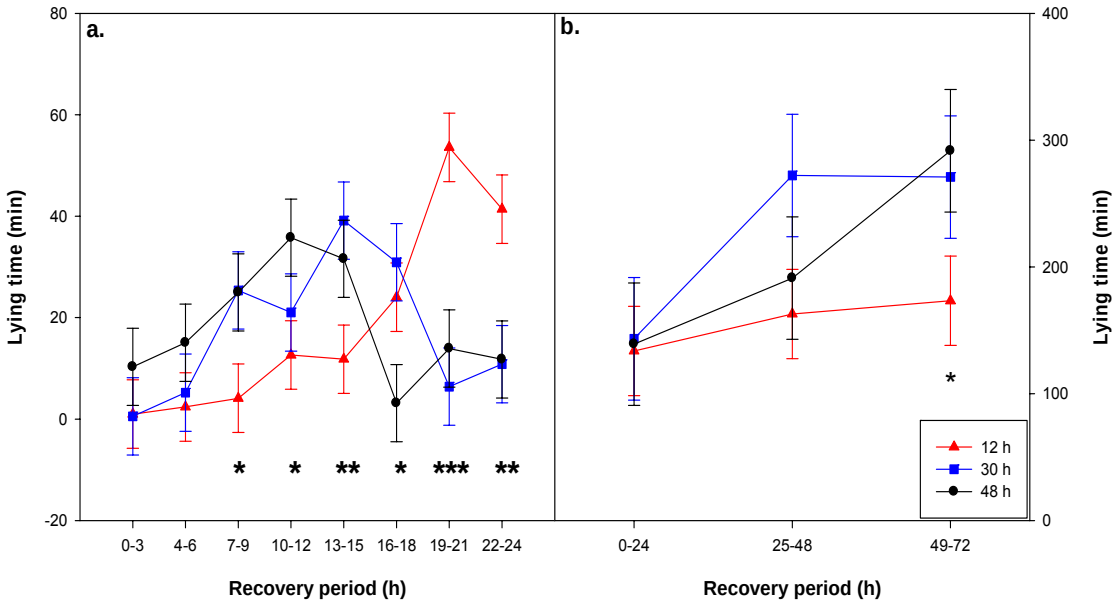


Figure 7ab Changes in lying time during (a) the initial 24 h of recovery and (b) the 72 h of recovery following 12, 30 or 48 h of transport.

Lying time was also analysed over the three 24 h periods of the recovery phase and there were significant effects due to period ($P<0.01$) and the interaction replicate x period ($P<0.001$). There were no differences in lying time between the transport duration treatments during any of the three 24 h recovery periods (Figure 7b). The time spent lying during the initial 24 h period tended to be lower than that observed in the subsequent periods.

3.5 Effect of transport duration on post-transport water consumption

Water consumption during the initial 24 h (8 x 3 h periods) and the full 72 h (3 x 24 h periods) of recovery was analysed on group basis. The water consumption pattern over the initial 24 h is shown in Figure 8a. Sheep transported for the longer durations of 30 h and 48 h drank significantly more than the 12 h duration group in the first 3 h of recovery, and thereafter the differences between the duration treatments were minimal. The largest differences between the transport duration treatments over the 72 h recovery period occurred during the initial 24 h and after that the water consumption levels were similar (Figure 8b).

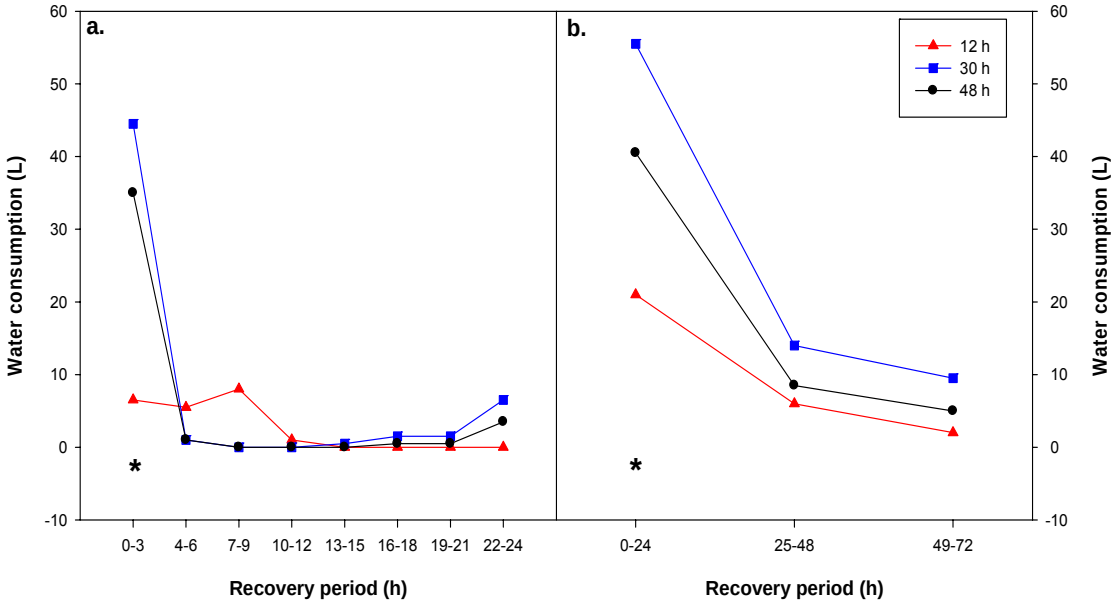


Figure 8ab Changes in post-transport water consumption during (a) the initial 24 h of recovery and (b) the 72 h of recovery following 12, 30 or 48 h of transport

4. Discussion

The results of this experiment indicate that mature healthy sheep that have not had restricted access to food or water prior to transport can tolerate transport up to 48 h without any major compromise to their welfare. These results generally support those of the earlier cattle transport duration experiment (Ferguson et al 2006) where some of the key physiological indicators were clearly influenced by transport duration however, the changes were not extreme and still within the normal expected physiological ranges for sheep (eg. Kaneko et al 1997).

During livestock transport, particularly over long durations (>24 h), it is the physiological states of dehydration and fatigue that are of most concern from an animal welfare perspective. In this experiment, as journey duration increased from 12 to 48 h there was a commensurate increase in measures of haemoconcentration and urine concentration. It is difficult to compare the result obtained here with other published data because there have been very few sheep transport duration studies. Furthermore, in these few studies (eg. Knowles et al 1993, 1994, 1995; Broom et al 1996), the transport duration treatments are generally less (≤ 24 h) than those examined in the present experiment.

Knowles et al (1994) transported lambs 24 h and observed slightly lower serum total protein levels (72.3 g/L) than those observed here after 30 and 48 h of transport (72.9 - 74.8 g/L). On the other hand, the serum osmolality values for the longer journeys (298.6 - 302.6 mOsmol/L) were very similar to the 300 mOsmol/L reported by Knowles et al (1994). In a subsequent study where sheep (age not defined) were transported for 24 h, Knowles et al (1995) observed lower serum measures of total protein concentration (~ 67.9 g/L) and osmolality (~ 293.1 mOsmol/L) than their earlier study. In another UK study by Parrot et al (1998) where 50 kg lambs were transported for 31 h with a 1 h rest stop after 14 h where they were unloaded and had access to feed and water, the serum osmolality were lower at the conclusion of the journey compared to the levels measured pre-transport. Very few animals drank during the rest period and these authors concluded that dehydration was not likely to be an issue in sheep during journeys of 31 h.

The results from food and water deprivation studies are also informative in this context even though animals were not transported. Jacob et al (2006) deprived lambs of feed and water for 48 h and reported the serum osmolality levels rose to approximately 297 mOsmol/L, which is similar to the present results. They also measured urine composition and concentration and after 48 of feed and water deprivation, urine SG was approximately 1.036. This is comparable to levels observed here after 12 h of transport (1.034) and much lower than those found after 30 or 48 h of transport (1.060). The disparity here could be associated with experimental differences in levels of water consumption prior to commencement of the transport or feed and water deprivation treatments. Furthermore, transport is likely to be psychologically more demanding than feed and water deprivation and Parker et al (2004) have shown that stimulation of the HPA axis and the subsequent secretion of cortisol can play a role in maintaining water balance through its suppression of the renin-angiotensin-aldosterone axis. This is turn, is likely to influence diuresis and urine concentration. It is also important to highlight that urine SG, whilst informative, has limitations as an indicator of hydration status. Concentrating the urine is an adaptive response to reduced fluid intake and therefore urine SG is probably a more useful indicator of fluid intake rather than dehydration *per se* (Jacob et al 2006).

In the present study, haemoconcentration associated with increasing journey duration was apparent, but the level of dehydration even after 48 h could not be classed as being of clinical concern (eg. serum osmolality <300 mOsmol/L and total protein < 79 g/L). This outcome can largely be attributed to the ruminal reservoir of fluid which acts as a useful buffer during periods of water restriction (Knowles and Warriss 2000). That said, it was clear based on the water consumption patterns during initial 3 h of recovery that the animals transported for 30 h or 48 h were considerably thirstier than the 12 h group, but they rapidly rehydrated.

Transport for 30 and 48 h resulted in an increase in the creatine kinase (CK) levels but the opposite was observed for the 12 h treatment. The leakage of CK from muscle will be function of the intensity and duration of muscular exertion and the incidence of muscle trauma which can arise during normal handling. The reduction in CK over 12 h of transport may have been due to the combination of higher incidence of pre-transport muscular trauma and the shorter journey was less physically demanding for the animals.

Overall, the differences in CK between the transport treatments were relatively small as was the peak levels observed after transport which were quite similar to those reported by Parrot et al (1998). We also concur with Parrot et al (1998) that well-managed transport for moderate to long durations does not cause significant injurious effects in sheep.

It is also worth noting that the pre-transport differences between the transport duration treatments or transport duration x replicate groups for some of physiological measures did not have a large bearing on the results. This was determined by fitting the post-transport models with and without the pre-transport level as a covariate. The covariate was often significant but it did not alter the significance of the primary interaction of interest notably, transport duration x time.

From the behavioural data during transport, there was a trend for increased incidence of lying as the transport duration increased. However, the differences in lying behaviour during the recovery phase does not indicate that the animals transported over longer durations were more tired than the animals transported over the shorter duration of 12 h. This contrasts with the observations by Ferguson et al (2006) in cattle. Clearly, the greater tolerance of sheep to stand for long periods during transport can be attributed to their lighter mass compared to cattle. Knowles et al (1995) observed that following 24 h of transport, sheep displayed slight transient increases in feeding and drinking activity before quickly resuming normal patterns of behaviour. However it is worth noting that in the Knowles et al (1995) study, the stocking density during transport was sufficient enough to allow the animals to lie down. During the initial 24 h of recovery there appears to be three phases where there was very little difference in lying behaviour between the transport duration groups during the first 6 h followed by increased lying in the 30 and 48 h treatment groups during the subsequent 12 h followed by a reversal in the this trend during the remaining 6 h. This might suggest that the longer journey durations has influenced the priority given to lying during the initial recovery phase.

The plasma measure of protein catabolism (blood urea nitrogen) generally increased throughout the transport and recovery phases. However the changes were relatively small and well within the normal range for sheep. The increase in the ketone β-hydroxy butyrate (BHB) which is indicative of some lipid catabolism in response to 30 or 48 h transport duration was similar to that observed by Knowles et al (1995) after 24 h of transport.

The trends in the rectal temperature profiles reinforce the general view that loading and the initial stages of transport are the most stressful (Eldridge et al 1988, Warriss et al 1995, Broom et al 1996, Pettiford et al 2007). After 3-4 h of transport, rectal temperatures returned and remained at normal levels irrespective of journey duration, suggesting that the sheep had habituated to the journey conditions.
The haematology results showed that increasing transport duration did not elicit major changes in differential leucocyte or erythrocyte counts. Leucocyte numbers did increase with transport and this was largely associated with the stress-mediated neutrophilia. These increases were quickly dissipated during the recovery phase and by 48 h most had returned to pre-transport levels. These general trends align with those observed in the cattle transport duration experiment (Ferguson et al 2006). The differences in the

post-transport changes in the red blood cell counts between the duration treatments are difficult to explain. The measure provides some indication of haemoconcentration but it is perhaps less informative compared to serum osmolality. This stems from the fact the red blood cell counts can increase via the effects of psychological stress resulting in splenic production of new cells (Knowles and Warriss 2000)

Measurements of serum cortisol concentration are widely used in the assessment of an animal's stress response, particularly in relation to psychological stressors. The treatment mediated differences in cortisol levels observed in this study were generally not large. Furthermore, the levels on arrival were lower than those observed prior to transport. In this instance, it can be interpreted that transport up to 48 h has not elicited a major psychological effect on the animals. These results align with the general view from ruminant transport research that after an initial period of adjustment to the stressors associated with transport, sheep habituate to the transport conditions.

The effect of transport duration on liveweight lost was consistent with other published data. Wythes and Morris (1994) reviewed several experiments where liveweight was measured in sheep over different periods of food and/or water deprivation. They reported a mean weight loss after 12 and 48 h of 5% and 11 %, respectively, which is similar to the results (4.9 % - 12 h and 12.1 % - 48 h) in the present study. The rate of liveweight recovery during the 72 h post-transport recovery phase was relatively fast during the initial 24 h particularly for the 30 and 48 h transport groups but then tended to plateau. Consequently, after 72 h of recovery, the transport groups had only reached between 91.4 - 95.8% of their pre-transport liveweights. It took between 7 – 14 days after transport for the sheep to recover or exceed their pre-transport liveweights. Knowles et al (1993) transported lambs for 14 h and observed that the liveweight had not fully recovered to the pre-transport liveweight after 12.5 days post-transport. The rate of recovery is going to be influenced by a number of factors but in the present experiment, the daily handling and yard environment may not have been conducive to a normal rate of recovery.

Finally, it is pertinent to highlight that the differences between the replicates (ie. week) was a large source of variation for many of the physiological parameters. This reinforces the need for replication in animal studies but also flags a cautionary note when drawing conclusions based on a single cohort of animals.

5. Conclusions

Healthy mature sheep with no pre-transport feed or water curfew and transported in accordance with accepted good practice generally coped with transport durations up to 48 h. Whilst those on the longer journeys (especially 30 and 48 h), were more thirsty initially on arrival, the physiological and behavioural data indicates that they were not clinically compromised. Given these results, the revised maximum duration of 32 h with the option to extend to 38 h (if the animals are not displaying obvious signs of fatigue, thirst or distress and if the extension allows the journey to be completed within 38 h) under the draft Australian Model Code of Practice for the Land Transportation of Sheep, is conservative on animal welfare grounds for the class of animal examined in this study. Furthermore, these results also facilitate some confidence that animal welfare was not

inherently compromised by the 48 h duration existing in the code since 1983 in healthy mature sheep transported in accordance with good practice.

6. References

Broom, D.M., Goode, J.A., Hall, S.J.G., Lloyd, D.M and Parrott, R.F. (1996). Hormonal and physiological effects of a 15 hour road journey in sheep: comparison with the responses to loading, handling and penning in the absence of transport. *Br. Vet. J.* **152**:593-605.

Eldridge, G.A., Winfield, C.G. and Cahill, D.J. (1988). Responses of cattle to different space allowances, pen sizes and road conditions during transport. *Aust. J. Exper. Agric.* **28**:155-159.

Ferguson, DM, Pettiford, SG, Fisher AD, Lee, C, Paull DM, Lea, JM, Reed MT and Niemeyer DDO (2006). The effect of transport duration on indicators of cattle welfare. MLA Milestone Report Project AHW.055.

Jacob, R.H., Pethick, D.W., Clark, P., D'Souza, D.N., Hopkins, D.L. and White, J. (2006). Quantifying the hydration status of lambs in relation to carcass characteristics. *Aust. J. Exper. Agric*. **46**: 429-437.

Jones, G.E. and Mould, D.L. (1984). Adaptation of the guaiacol (peroxidase) test for haptoglobins in a microtitration plate system. *Res. Vet. Sci*. **37**:87-92.

Kaneko, J.J., Harvey, J.W. and Bruss, M.L. Eds. (1997). "Clinical Biochemistry of Domestic Animals" 5th Edition. (Academic Press, London, UK).

Knowles, T.G., Brown, S.N., Warriss, P.D., Phillips, A.J., Dolan, S.K., Hunt, P., Ford, J.E., Edwards, J.E. and Watkins, P E. (1995). Effects on Sheep of Transport by Road for up to 24 Hours. *Veterinary Record* **136**: 431-438.

Knowles, T.G. and Wariss, P.D. (2000). Stress physiology of animals during transport. In: "Livestock Handling and Transport" 2nd Edition. Ed T.Grandin, (CAB International, Oxon UK).

Knowles, T.G., Warriss, P.D., Brown, S.N. and Kestin, S.C. 1994. Long Distance Transport of Export Lambs. *Veterinary Record* **134**: 107-110.

Knowles, T.G., Warriss, P.D., Brown, S.N., Kestin, S.C., Rhind, S.M., Edwards, J.E., Anil, M.H. and Dolan, S.K. (1993). Long Distance Transport of Lambs and the Time Needed for Subsequent Recovery. *Veterinary Record* **133**: 286-293.

Parker, A.J., Hamlin, G.P., Coleman, C.J. and Fitzpatrick, L.A. (2004). Excess cortisol interferes with a principal mechanism of resistance to dehydration in *Bos indicus* steers. *J. Anim. Sci*. **82**: 1037-1045.

Parrott, R.F., Hall, S.J.G., Lloyd, D.M., Goode, J.A. and Broom, D.M. (1998). Effects of a Maximum Permissible Journey Time (31 H) on Physiological Responses of Fleeced and Shorn Sheep to Transport, With Observations on Behaviour During a Short (1 H) Rest-Stop. *Animal Science* **66**: 197-207.

Pettiford, S.G., Ferguson, D.M., Lea, J.M., Lee, C., Paull, D., Reed, M.T. and Fisher, A.D. (2007). The effect of loading practices and 6 h road transport on the physiological responses of yearling cattle. *Aust. J. Exper. Agric*. (in preparation).

SAS (1999). SAS/STAT User's Guide. Version 6, Fourth edition, Cary, N.C.: SAS Institute

Slocombe, L.L. and Colditz, I.G. (2005). Evaluating the stress of production in cattle using haptoglobin. 5th Annual International Colloquium on Animal Acute Phase Proteins, Dublin, Ireland.

Warriss, P.D., Brown, S.N., Knowles, T.G., Kestiin, S.C., Edwards, J.E., Dolan, S.K. and Phillips, A.J. (1995). Effects on cattle of transport by road for up to 15 hours. *The Vet. Rec.* **126**:319-323.

Overall progress of the project

The project continues to track well and is on schedule. The final experiment examining the interaction between curfew duration and transport duration in lambs is scheduled to commence in early April 2007.

Recommendations

Based on these results, the previous and revised maximum transport duration for mature healthy dry sheep as currently stated within draft Australian Model Code of Practice for the Land Transport of Sheep are acceptable on animal welfare grounds when animals are transported in accordance with best practice. The revised transport code is therefore conservative based on these results.

Milestone 9

Milestone report on completion of stage 2 (sheep) research. Animal welfare outcomes determined post-transport for different curfew and transport duration combinations. Recommendations provided on best practice transport management for curfew and duration combinations.

Abstract

The aim of this experiment was to quantify the effect of pre-transport food and water deprivation (curfew) on the response to transport in Merino lambs. One hundred and eighty Merino lambs aged 6-7 months were used for the experiment. Of these, detailed measurements were made on 120 focal ewe lambs. A factorial design was used comprising three pre-transport food and water deprivation treatments of 0, 12 and 24 h and two transport duration treatments of 12 and 24 h, and these were replicated twice over a period of 4 weeks. At the conclusion of their transport treatments, the lambs were placed in pastured paddocks with access to water for 72 h. Detailed measurements of liveweight, blood chemistry, haematology, body temperature, lying behaviour and water intake during recovery were recorded pre- and post-curfew, pre-transport and 0, 24, 48 and 72 h post-transport. Pre-transport curfews resulted in a significant reduction in liveweight and significant increase in serum cortisol, blood urea nitrogen and albumin concentrations. Total and differential white cell counts were also significantly affected. In the analysis of the pre- and post-transport data, several significant interactions were found for many of the measures. Notable here was the interaction between curfew treatment x transport treatment x time (sampling) which was was significant for many of the blood measures (blood urea nitrogen, total protein, albumin, β-hydroxybutyrate, cortisol, white blood cell count, neutrophil and lymphocyte counts and neutrophil:lymphocyte ratio), liveweight and faecal score. The combined periods of curfew and transport were additive for their effects, particularly for liveweight loss observed immediately post-transport. This trend was less evident however for most of the other physiological measures, particularly those that indicate hydration status. Lambs that were curfewed (12 or 24 h) spent more time lying during journeys of 24 h compared to non-curfewed animals. Curfew duration and transport (but not transport duration) resulted in a decrease in faecal scores reflecting a decrease in faecal moisture levels but did not affect microbiological characteristics of the faeces. In conclusion the results of this investigation indicate pre-transport feed and water withdrawal did not adversely affect animal welfare, but simply added to the overall feed and water deprivation period and its associated effects. Furthermore, subjecting healthy, grass-fed lambs to pre-transport periods of food and water deprivation (12 and 24 h compared with 0 h) prior to transport for 12 or 24 h did not enhance the capacity of the animals to cope with transport.

Project objectives

1. Determine the contribution of handling, loading and initial transport processes on the stress responses of cattle to road transport.
2. Determine the animal welfare outcomes of yearling cattle transported under controlled conditions for 6, 12, 30 or 48 hours from farm to feedlot entry.
3. Determine the animal welfare outcomes of mature sheep (eg. live export trade) transported under controlled conditions for 12, 30 or 48 hours.

4. Determine the impact of pre-transport curfews for 0, 12 or 24 h on the welfare outcomes of 12 and 24 h of transport in yearling cattle and mature sheep
5. Develop recommendations on best practice yearling cattle and cast for age sheep transport management for optimal welfare and productivity.

Success in achieving milestone

THE EFFECT OF PRE-TRANSPORT PERIODS (0, 12 AND 24 H) OF FOOD AND WATER DEPRIVATION ON THE RESPONSE TO 12 AND 24 H OF TRANPORT IN MERINO LAMBS

Drewe Ferguson, Dominic Niemeyer, Caroline Lee, David Paull, Jim Lea, Matt Reed and Andrew Fisher

1. Introduction

The standards of husbandry and welfare practiced during livestock production are becoming important factors influencing consumer perceptions in many markets. Clearly, the use of welfare-unfriendly livestock transport practices has the potential to downgrade product quality through reductions in meat quality and increased bruising. Furthermore, the use of practices that initiate market and public concerns that are unable to be adequately addressed may cause damage to the image and market access of Australian livestock products.

There is little scientific evidence that the welfare of cattle and sheep trucked under Australian conditions is superior or equivalent to that of animals transported by our competitors and international trading partners. Further, there are some marked differences in our conditions and practices compared with those of others. If Australian practices and regulations are going to be different of necessity from those of other trading blocs, then we may need objective evidence that the animal welfare outcomes are equivalent and acceptable. Consequently this project was designed to develop scientifically defensible quantification of the animal welfare outcomes of specific Australian livestock transport practices. The research reported here represents the fourth in a series of investigations examining the animal welfare outcomes of land transport practices for cattle and sheep.

During transport, it is inevitable that there will be short to moderate periods of restricted access to food and water. Whilst food and water deprivation will normally occur during transport, the period of deprivation can be substantially extended if animals undergo a curfew prior to transport. Curfew is the generic term used in livestock industries for the practice of enforced food or food and water deprivation prior to transport, sale or slaughter. Curfews are applied because of the demands and selling conditions of transport operators, livestock buyers and abattoir management, respectively. Curfews are typically 6 – 12 h in duration and the primary reason for their use is to reduce the gastrointestinal volume (empty out) prior to transport, thus reducing the total amount of excreta in trucks and the level of faecal soiling on animals.

Transport operators have advocated that pre-transport curfews enable the animals to travel better. One of the primary benefits observed by livestock transporters was the

reduction in number of animals (primarily cattle) that go down during the journey. The risk of bruising and injury increases considerably when animals go down (Tarrant and Grandin 2000) and drivers are required to encourage these animals back to their feet which in turn, may cause additional stress in both the downed animal and others in the truck. In lambs the risk of smothering and death increases considerably if animals go down and are unable to stand. Regular stops to attend to downed animals will also prolong the transport duration.

There is very little published data corroborating these anecdotal views from the transport industry although it is clear that non-curfewed animals, particularly cattle, will produce more excreta during transport (Gregory et al 2000). Whilst the volume of excreta and indeed the design and construction of the stockcrate floor contribute to losses of balance and slippage, it is pertinent to highlight that stocking density and driving events (eg. braking, cornering) are also major factors in this context (Eldridge 1988, Tarrant et al 1992, Cockram et al 2004).

Another driver for the implementation of pre-transport curfews, especially for lambs destined for slaughter, is to reduce the level of faecal contamination on the wool/pelts. Increased faecal matter on the animal increases the risk of contamination of the carcass during slaughter and dressing (Pointon et al 2006). Of particular relevance here, is the risk of contamination by enteric pathogens such as *E.coli* O157 and *Salmonella spp*. Paradoxically, whilst curfews reduce the risk of faecal soiling and contamination on animals, they may also result in increased shedding of these bacteria (Pointon et al 2006). Further investigation of the countervailing effects of curfews in relation to food safety is required.

No clear conclusions can be drawn with regard to the interaction between pre-transport food and water deprivation and the response to transport, particularly long-haul transport, as there is a paucity of published data. This investigation was undertaken in view of the lack of scientific data to support the anecdotal views from livestock transporters that periods of food and water deprivation prior to loading improve the capacity of lambs to cope with transport. Furthermore, the effects of pre-transport curfews and transport on the shedding of enteric pathogens were also quantified by Food Science Australia.

2. Materials and Methods

The experiment was approved by the CSIRO Livestock Industries FD McMaster Animal Ethics Committee (Approval No. 07/05).

2.1 Lambs

One hundred and eighty Merino lambs were used in the experiment. The lambs were approximately 6-7 months of age and comprised both ewes and wethers. Of the total, there were 120 focal animals (all ewes) where detailed measurements were recorded. Prior to the experiment all the lambs were run as one group and maintained on improved temperate pastures (2351 kg DM/Ha). Eight days before the commencement of the experiment, the focal lambs were allocated to their 12 curfew duration x transport duration x replicate groups (n = 10/group) stratified for liveweight. The mean liveweight of the focal lambs at treatment allocation was 26.9 ± 3.0 kg. Each group plus an

additional 20 – 22 lambs were moved to a 0.8 ha improved pastured plot where they remained until the completion of the 72 h post-transport recovery phase. The additional lambs were used as fillers on the truck to ensure that the stocking density complied with the code for this weight class of stock. The mean herbage mass across the 12 plots at the beginning and near the end of the experiment was 4239 kg DM/Ha and 2934 kg DM/Ha, respectively.

The experiment was conducted in April – May 2007 where the ambient temperature ranged from 0.4 – 23.3^{o}C and the relative humidity ranged from 37-99%. During the period, a total of 110 mm of rain was recorded. These data were extracted from the CSIRO Armidale weather station records.

2.2 Experimental design and procedures

A factorial design was used comprising three pre-transport curfew durations (ie. food + water deprivation) of 0, 12 and 24 h and two transport durations of 12 and 24 h, and these were replicated twice over a period of 4 weeks. The lambs were transported on the CSIRO stock truck (16 t fixed chassis vehicle) over a route that included highway and secondary roads (no unsealed roads). The journey length was approximately 5.5 h in duration. This journey was repeated twice and four times for the 12 and 24 h journey duration treatments, respectively. Drivers either changed over or took a 30-min break between journey runs. For each journey duration, the journeys were undertaken to either commence during the day (8 am departure) or evening (8 pm departure), with a 50:50 balance between morning and eveing departures within each journey duration treatment. During this time the lambs remained on the vehicle.

During each week, three curfew/transport treatment groups were trucked. At approximately 1.5 h prior to the commencement of the curfew treatments, the lambs were yarded. The 10 focal lambs were weighed, fitted with vaginal temperature loggers, IceTag^{TM} behavioural monitors and sampled for blood. A faecal sample (approx 5 g) was manually collected and scored for consistency on a 1 – 5 scale (1 = normal free faecal pellets and 5 = watery diarrhoea). The descriptions of each score are detailed in Appendix 1. The focal and filler lambs were then placed in a yard to commence their curfew treatment.

At the conclusion of their curfew duration, the lambs were reweighed and further blood and faecal samples were taken before the animals were loaded onto the truck to commence their transport duration treatments. The lambs were also visually assessed for dirtiness based on a 0 – 3 scale (0 = clean and 3 = very dirty) in eight regions on the body (head, neck, midback, rump and tail, flank and upper hind leg, lower hind leg, brisket and upper fore leg and lower fore leg). A diagram depicting the body regions is attached as Appendix 2.

The animals were placed in the front pen of the stock crate at a stocking density of 0.19 m^2/head which complied with the welfare transport code for this liveweight class of sheep (Figure 1). The vehicle was fitted with a GPS logger and a temperature and humidity logger (Tiny Tag plus TGP1500 loggers, Omni Instruments, Scotland - temperature range -30 to +50^{o}C and relative humidity 0 to 100%) was placed in the stockcrate.

Figure 1 Lambs in stock crate just prior to commencement of the journey.

After completing their journeys, the lambs were unloaded at a second set of yards to commence their 72-h recovery period. Upon arrival, the lambs were weighed and blood and faeces were collected and this was repeated 24, 48 and 72 h after arrival. They were also visually assessed for dirtiness. A photograph of the floor of the truck at the conclusion of the journey was also taken (Appendix 3).

During the 72-h recovery period, the lambs returned to their pastured plots. The water supply to each plot was monitored (Hobo® Event logger, Onset Computer Corporation, Bourne, MA 02535, USA) to measure the daily water utilisation by each group during the recovery phase.

After the 72-h recovery period, the IceTag^{TM} behavioural monitors and rectal temperature loggers were removed and the animals were placed in a new paddock.

2.3 Blood sampling and biochemical measurements

Each animal was blood sampled via jugular venipuncture on 6 occasions; prior to and on completion of the curfew period and 0, 24, 48 and 72 h post-transport. The sample taken on completion of the curfew treatment was also defined as the pre-transport sample. Two blood samples were taken at each time point (6 ml EDTA and 10 ml serum vacutainers). An aliquot of whole blood was analysed for the haematology variables haemoglobin (HGB), red cell counts (RCC), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelet count (PC), white blood cells count (WBC) and differential cell counts of neutrophils (NEU), lymphocytes (LYM), monocytes (MON), eosinophils (EOS), basophils (BAS), using a Cell-Dyn Haematology Analyser 3500R (Abbott Diagnostics, CA, USA).

An aliquot of blood was centrifuged and the resultant serum was harvested and frozen (-20^{o}C) for subsequent analysis of blood chemistry.

The concentrations of cortisol, blood urea nitrogen, total protein, albumin, creatine kinase, β-hydroxy butyrate and haptoglobin were determined on the serum samples. Cortisol was determined using a Spectria Cortisol RIA (Orion Dianostica, Espoo, FIN). An OLYMPUS AU400 automated clinical analyser was used to analyse the blood urea nitrogen (BUN), β-hydroxybutyrate (BHB), creatine kinase (CK) and total protein (TP) and albumin (ALB) concentrations. Osmolality (OSMOL) was also measured, using a vapour pressure osmometer (Wescor, 5500). The acute phase protein haptoglobin (HAPT) was determined in plasma by a variation of the method of Jones and Mould (1984). The assay was modified to account for the effect of free haemoglobin due to haemolysis (Slocombe and Colditz, 2005).

2.4 Faecal sampling and microbiology

Faecal samples were manually collected prior to and on completion of the curfew and 0, 24, 48 and 72 h post-transport. The faeces were scored for consistency according the scale described above. On some occasions it was not possible to collect faeces, so swabs of the rectal wall were collected. The faecal samples and swabs were placed in

50 ml sterile containers and these were sent to Food Science Australia for analysis of enteric pathogen levels *E.coli* O157 and *Salmonella*. The methodologies applied are detailed in the FSA report which is attached as Appendix 4.

2.5 Body temperature
On the day of transport, focal sheep were fitted with vaginal temperature loggers. Temperature loggers (Thermochron iButton, Maxim Integrated Products, USA) were secured to sheep CIDRs. Body temperature was recorded every 4 min until the harnesses and probes were removed at 72 h post-transport.

2.6 Behavioural assessment
IceTag™ behavioural monitors (IceRobotics, Midlothian, Scotland, UK) were secured to the left fore leg of each animal via velcro and duct tape according to the manufacturer's instructions (Figure 2). These monitors recorded whether the animal was lying, standing or active (walking, moving) and measurements were recorded every second.

Figure 2 Fitting of IceTag™ behavioural monitor to left fore leg.

2.7 Statistical Analysis
Several measures were transformed to stabilise variances prior to analysis. The GLM and MIXED model procedures in SAS (SAS Institute Inc., Cary, NC, USA.) were used to analyse the data. In determining the effects of curfew duration, the GLM procedure was used where the initial model contained the fixed effects of curfew treatment and replicate plus the interaction. A model comprising the terms curfew treatment, transport treatment, replicate and time (pre-transport, 0 h post-transport, 24 h post-transport, 48 h post-transport and 72 h post-transport) and interactions plus a random term for animal was used to quantify the main fixed effects of curfew and transport duration. These models were applied for the liveweight and blood measurements. Lying behaviour during post-transport recovery was also analysed used the MIXED model procedure. Two analyses were conducted. The first analysis examined the changes in lying behaviour over the initial 24 h (8 periods of 3 h) of recovery. The second analysis examined these changes over the three consecutive 24-h periods of recovery. The terms in the model were similar to those described above with the exception that recovery period was included instead of sampling time. The water consumption over the three 24-h periods of recovery was analysed using the GLM procedure in SAS. For all the analyses, non-significant interactions were sequentially removed to reveal the final model.

3. Results

3.2 Pre-transport measurement of blood chemistry and haematology
The pre-curfew means for the blood chemistry and haematology variables (Table 1) generally conformed to the accepted normal clinical ranges for sheep (eg. Kaneko et al 1997). There were some parameters such as BUN and BHB where the levels were lower than the normal expected range but for these measures, it would be more concerning if the levels exceeded the normal upper limit.

Table 1 Pre-curfew means and normal clinical means or ranges for blood parameters

Blood Parameter	Mean	Normal Range or Mean ± sd*
Blood urea nitrogen (BUN mg/dL)	4.96	8.0 – 20.0
Total protein (TP g/L)	60.37	60.0 – 79.0
Albumin (Alb. g/L)	26.74	26.7 – 36.8
Creatine kinase (CK U/L)	238.42	7.7 – 101.0
β-hydroxy butyrate (BHB mmol/L)	0.28	0.55 ± 0.04
Cortisol (nmol/L)	63.37	62.0 ± 10.0
Osmolality (Osmol. mOsmol/L)	292.23	282-297
Haemoglobin (HGB g/L)	114.35	90.0 – 140.0
Red cell count (RCC x 10^{12}/L)	10.73	8.0 – 16.0
Haematocrit (HCT %)	0.35	0.24 – 0.50
Mean corpuscular vol. (MCV fL)	32.83	23.0 – 48.0
Mean conc. HGB in red cells (MCHC g/L)	328.05	310.0 – 380.0
Mean corpuscular HGB (MCH %)	10.76	34.0 – 38.0
Platelets count (PC x 10^9/L)	677.54	250.0 – 750.0
White blood cell count (WCC x 10^9/L)	6.06	4.0 – 12.0
Neutrophils (NEU x 10^9/L)	2.56	0.7 – 6.0
Lymphocytes (LYM x 10^9/L)	2.48	2.0 – 9.0
Mononcytes (MON x 10^9/L)	0.85	0 – 0.75
Eosinophils (EOS x 10^9/L)	0.05	0 – 1.0
Basophils (BAS x 10^9/L)	0.11	0 – 0.4

3.1 Effect of curfew duration on physiological measures

(i) Liveweight

There was a significant effect of curfew duration on liveweight ($P<0.01$), where there was a 4.6% and 7.8% reduction following 12 h and 24 h of food and water deprivation, respectively (Table 3, Figure 3). A significant interaction ($P<0.05$) between curfew duration x replicate was also observed.

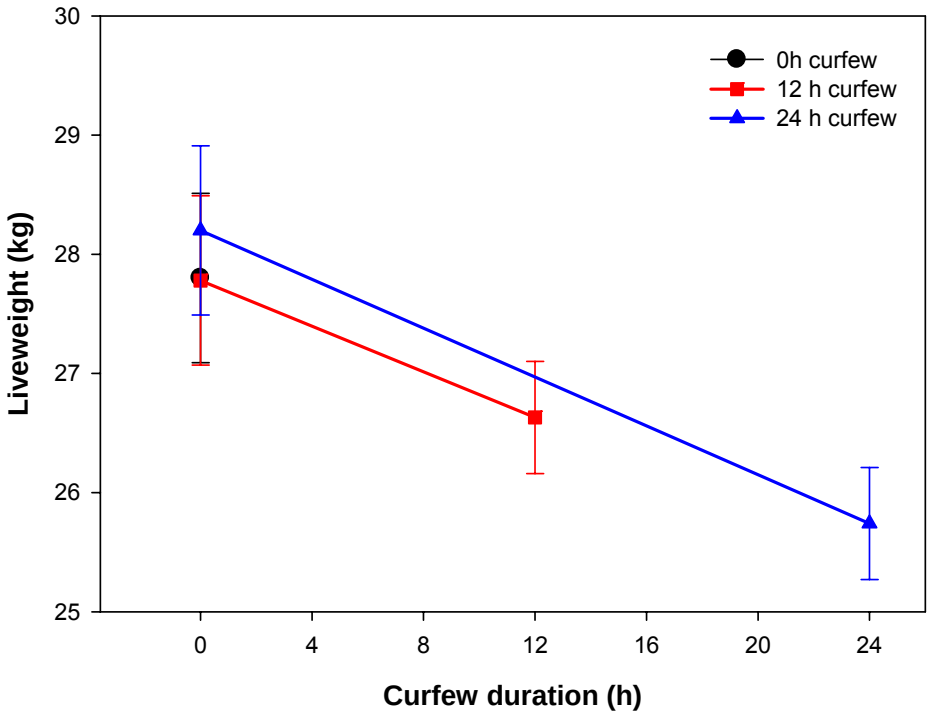


Figure 3 Effect of curfew duration on liveweight

(ii) Hydration measures
The blood measures of total protein and albumin concentration, haematocrit % and osmolality provide an indication of the animal hydration status. Prior to the commencement of the curfew treatments, significant differences were observed between the curfew treatments (TP) and replicates (OSMOL). The differences were quite small in magnitude. For example, the pre-curfew least square means for TP were 65.35, 58.57 and 57.20 for the 0, 12 and 24 h curfew treatments, respectively. With the exception of serum albumin, curfew duration had no effect on the haemoconcentartion measures (Table 2). Albumin levels were significantly higher ($P<0.01$) for the 12 and 24 h treatments compared to the control 0 h treatment.

(iii) Creatine kinase (CK)
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. Serum CK levels were significantly higher ($P<0.001$) for the 12 and 24 h treatments compared to the 0 h curfew group (Table 2). It is pertinent to point out here that unlike the 0 h curfew group, the 12 and 24 h groups were handled and sampled twice (pre- and post-curfew) and this may have led to the increase in CK levels rather than the direct effects of the curfew duration *per se*.

(iv) Cortisol
The serum cortisol concentrations increased with curfew duration ($P<0.001$). However, the differences between the treatments were only significant for the contrast between the 0 h and both the 12 and 24 h treatment groups (Table 2).

Table 2 Least square means ± sem for curfew treatment, replicate and significance of the interaction for plasma concentrations of blood urea nitrogen (BUN), total protein (TP), albumin (ALB), creatine kinase (CK), β-hydroxybutyrate (BHB), cortisol (CORT) and osmolality (OSMOL).

Main Effects	**BUN** (mg/dL)	**TP** (g/L)	**ALB** (g/L)	**CK*** (U/L)	**BHB** (mmol/L)	**CORT*** (nmol/L)	**OSMOL** (mOsmol/L)
Curfew (C)[#]							
0 h	5.20[a]	65.35	27.10[a]	196.72[a]	0.45	47.54[a]	295.63
12 h	6.35[b]	67.01	28.67[b]	335.14[b]	0.45	66.11[b]	292.49
24 h	7.75[c]	67.43	29.10[b]	270.09[b]	0.46	79.58[b]	293.76
sem	0.21	0.83	0.41	-	-	-	0.99
Significance	*P<0.001*	*ns*	*P<0.01*	*P<0.001*	*ns*	*P<0.001*	*P=0.08*
Replicate (R)							
1	6.49	66.10	27.97	260.92	0.45	63.94	289.73
2	6.38	67.09	28.62	261.35	0.45	62.08	292.17
sem	0.17	0.68	0.33	-	-	-	0.81
Significance	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*P<0.05*
Interaction							
C x R	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*

*backtransformed least square means shown
Means without common superscripts are significantly different (P<0.05)

(v) Haematology

The results for a subset of haematology measures are presented in Table 3. The interaction between curfew duration x replicate was significant for RCC only (P<0.05). The LS means for this interaction (results not shown) did not reveal any clear trend with increasing duration and in general, the differences were quite small. For WCC and the differential cell counts of NEU and LYM and the ratio NEU:LYM, a significant effect of curfew duration was found. The WCC and NEU counts after 12 h curfew were significantly higher than either the 0 h or 24 h treatments (P<0.001). LYM counts tended to decline with increasing curfew duration but the differences were only significant for the contrasts between the 0 h and both the 12 and 24 h curfew treatments. The NEU:LYM was highest for the 12 h curfew duration.

(vi) Haptoglobin

The serum levels of the acute phase protein haptoglobin were significantly influenced by the interaction curfew duration x replicate (P<0.05, Table 3). Focusing on the specific effect of curfew duration, the haptoglobin levels were higher in the 24 h treatment relative to the 0 or 12 h curfew treatments.

Table 3 Least square means ± sem for curfew treatment, replicate and significance of the interaction for a subset of blood haematology measures (red cell count – RCC, Haematocrit – HCT, white blood cell count – WCC, neutrophils – NEU, lymphocytes – LYM, neutrophils:lymphocytes ratio – NEU:LYM), haptoglobin (HAPT) and liveweight (LWT).

Main Effects	RCC ($x10^{12}$/L)	HCT (%)	WCC* ($x10^{9}$/L)	NEU* ($x10^{9}$/L)	LYM* ($x10^{9}$/L)	NEU:LYM*	HAPT*	LWT (kg)
Curfew (C)#								
0 h	10.77	34.96	5.95[a]	2.34[a]	2.35[a]	1.00[a]	0.14[a]	27.80[a]
12 h	10.93	35.29	7.20[b]	4.02[b]	1.90[b]	2.12[b]	0.15[a]	26.63[ab]
24 h	11.04	35.58	5.44[a]	2.42[a]	1.87[b]	1.29[c]	0.17[b]	25.74[b]
sem	0.20	0.42	-	-	-	-	-	0.47
Significance	*ns*	*ns*	*P<0.001*	*P<0.001*	*P<0.01*	*P<0.001*	*P<0.001*	*P<0.01*
Replicate (R)								
1	10.62	34.77	5.89	2.60	1.97	1.32	0.15	26.30
2	11.21	35.79	6.44	3.09	2.09	1.48	0.15	27.14
sem	0.17	0.35	-	-	-	-	-	0.38
Significance	*P<0.05*	*P<0.05*	*ns*	*P<0.05*	*ns*	*ns*	*ns*	*ns*
Interaction								
C x R	*P<0.05*	*ns*	*ns*	*ns*	*ns*	*ns*	*P<0.05*	*P<0.05*

*backtransformed least square means shown
Means without common superscripts are significantly different (P<0.05)

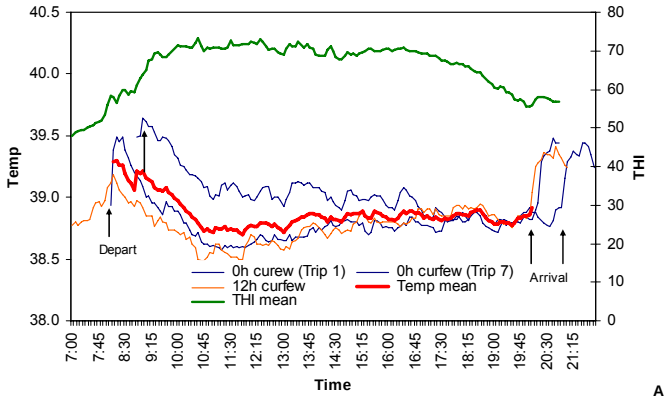
(vii) Blood urea nitrogen (BUN) and β-hydroxybutyrate (BHB)
Changes in BUN and BHB provide an indication of metabolic changes, specifically protein and lipid catabolism, respectively. Prior to the commencement of the curfew, significant differences (P<0.05) between the curfew treatments (BUN and BHB) and replicates (BUN) were observed. Once again these differences (BUN 0, 12 and 24 h - 5.20, 5.09 and 4.59 mg/dL; BHB 0, 12 and 24 h - 0.45, 0.48 and 0.47 mmol/L) were relatively small. For the blood metabolic markers BUN and BHB, the effect of curfew duration was only significant in the case of BUN (P<0.001) (Table 1). As the curfew duration increased there was a commensurate increase in BUN levels.

3.2 Effect of curfew and transport duration on physiological measures during and after transport

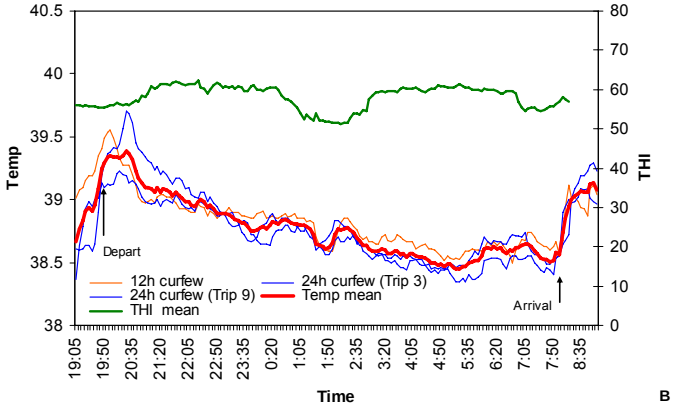
The rectal temperature profiles during the transport phase for 12 h and 24 h transport treatments are presented in Figure 4. The profiles are consistent with previous results in that the process of loading and initial stages of transport invoked an increase in temperature followed by a reduction to more basal levels. The THI data indicates that the on-board conditions were generally comfortable for the animals (THI $\leq$ 70) during the majority of the journey. However, there were short periods (mid-morning and mid-afternoon) where the THI increased to levels between 70-75 and the animals may have experienced some mild heat stress although this was not reflected in body temperature during these periods.

A significant four-way interaction between curfew duration x transport duration x replicate x time was found for a number of measures (liveweight, RCC, HCT and the serum concentrations of BUN, TP and BHB) (Table 4). This interaction generally showed that the key effect of interest, namely the magnitude of the interaction between curfew treatment x transport treatment x time varied between replicates. Variation between

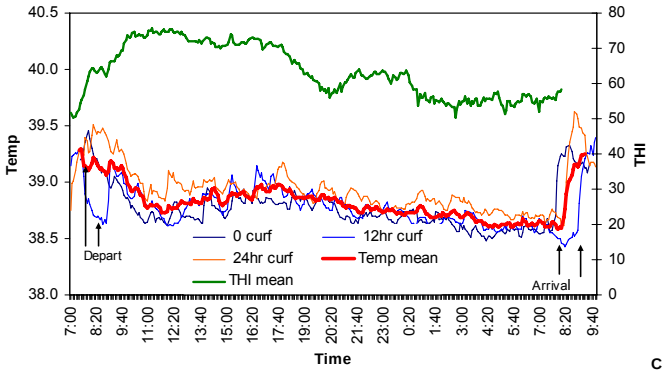
replicates was expected. In view of this, and in the interests of simplicity, emphasis was given to the three-way interaction between curfew treatment x transport treatment x time.



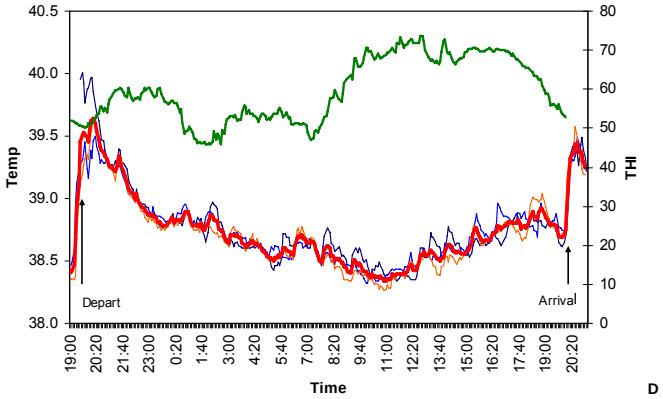
12 Hr – am Departure



12 Hr – pm Departure



24 Hr – am Departure



24 Hr – pm Departure

Figure 4 Rectal temperature profiles and the temperature-humidity index (THI) during: a. 12 h of transport (am departure), b. 12 h of transport (pm departure), c. 24 h of transport (am departure) and d. 24 h of transport (pm departure)

Table 4 Least square means ± sem for curfew treatment, transport duration, replicate and time and significance of the interactions for serum concentrations of blood urea nitrogen (BUN), total protein (TP), albumin (ALB), creatine kinase (CK), β-hydroxybutyrate (BHB), cortisol (CORT) and serum osmolality (OSMOL)

Main Effects	**BUN** (mg/dL)	**TP** (g/L)	**ALB** (g/L)	**CK*** (U/L)	**BHB*** (mmol/L)	**CORT*** (nmol/L)	**OSMOL** (mOsmol/L)
Curfew duration (C)#							
0 h	4.51[a]	62.16	27.13	178.30[a]	0.48[a]	69.01	293.13
12 h	5.68[b]	63.16	27.74	209.85[b]	0.47[a]	66.62	292.06
24 h	5.68[b]	62.27	28.06	195.06[ab]	0.46[b]	75.48	292.60
sem	0.09	0.59	0.35	-	-	-	0.50
Significance	*P<0.001*	*ns*	*ns*	*P<0.01*	*P<0.001*	*ns*	*ns*
Transport duration (T)							
12 h	4.75	62.21	27.51	187.01	0.48	69.13	292.18
24 h	5.83	62.85	27.77	201.19	0.45	71.43	293.02
sem	0.08	0.47	0.28	-	-	-	0.41
Significance	*P<0.001*	*ns*	*ns*	*P=0.06*	*P<0.001*	*ns*	*ns*
Replicate (R)							
1	5.31	61.71	27.51	193.83	0.48	65.82	292.24
2	5.27	63.35	27.78	194.11	0.46	75.02	292.96
sem	0.08	0.47	0.28	-	-	-	0.41
Significance	*ns*	*P<0.05*	*ns*	*ns*	*P<0.001*	*P<0.05*	*ns*
Time#							
Pre-transport	6.43[a]	66.59	28.29[a]	261.13[a]	0.45[b]	63.01[b]	290.95[a]
0 h post-transport	6.26[a]	58.39	28.05[a]	246.23[a]	0.50	81.25[a]	296.39
24 h post-transport	4.50[b]	62.82[a]	27.30	190.87	0.46[ab]	85.26[a]	291.20[a]
48 h post-transport	4.61[b]	63.41[a]	27.67	139.85	0.47[a]	69.28[b]	293.38
72 h post-transport	4.64[b]	61.44	26.91	160.00	0.45[b]	56.67	291.07[a]
sem	0.08	0.42	0.22	-	-	-	0.58
Significance	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.001*
Interactions							
C x T	*P<0.05*	*ns*	*ns*	*P<0.01*	*P<0.05*	*ns*	*ns*
C x R	*ns*	*ns*	*ns*	*P=0.05*	*ns*	*ns*	*ns*
C x Time	*P<0.001*	*P=0.06*	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.001*	*P=0.09*
T x R	*P<0.001*	*P<0.05*	*P<0.01*	*P<0.05*	*P<0.01*	*ns*	*P<0.01*
T x Time	*ns*	*P<0.001*	*P<0.05*	*ns*	*P<0.001*	*P<0.01*	*P=0.09*
R x Time	*P<0.001*	*P<0.001*	*P<0.001*	*ns*	*P<0.001*	*P<0.01*	*ns*
C x T x R	*ns*	*P<0.05*	*P<0.05*	*P<0.05*	*P<0.001*	*ns*	*ns*
C x T x Time	*P<0.001*	*P<0.01*	*P<0.05*	*ns*	*P<0.05*	*P<0.01*	*ns*
C x R x Time	*P<0.001*	*ns*	*P<0.05*	*P<0.001*	*P<0.01*	*P<0.05*	*P<0.01*
T x R x Time	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.01*	*ns*	*P<0.05*	*ns*
C x T x R x Time	*P<0.001*	*P<0.001*	*ns*	*ns*	*P<0.001*	*ns*	*ns*

*backtransformed least square means shown
Means without common superscripts are significantly different (P<0.05)

(i) Liveweight

There was a significant difference in the pre-transport liveweights as a consequence of the curfew treatments (Table 5, Figure 4). For the 24 h transport groups, lambs not

curfewed were significantly heavier than those from the 12 and 24 h curfew treatments. For the 12 h transport treatment, the group curfewed for 24 h had significantly lighter pre-transport liveweights relative to the other two curfew durations.

The significant interaction between curfew treatment x transport treatment x time (P<0.001) was influenced by differences throughout the post-transport phase. On arrival, percentage loss in liveweight ranged from 5.3 – 9.8 %. The magnitude of the loss was inversely proportional to the curfew duration for the 24 h transport groups. For example, the liveweight lost after 24 h of transport was 9.8, 8.8 and 8.3 % for the 0, 12 and 24 h curfew treatments, respectively. This trend was not evident for the 12 h transport treatment. From Figure 5 it can be seen there are two distinct treatment group clusters in liveweight on arrival. These clusters are associated with the total curfew + transport time (ie. total time of food and water deprivation). On arrival (0 h), there was no difference in the liveweight for the groups where the total curfew + transport time ranged between 12 – 24 h or 36 – 48 h.

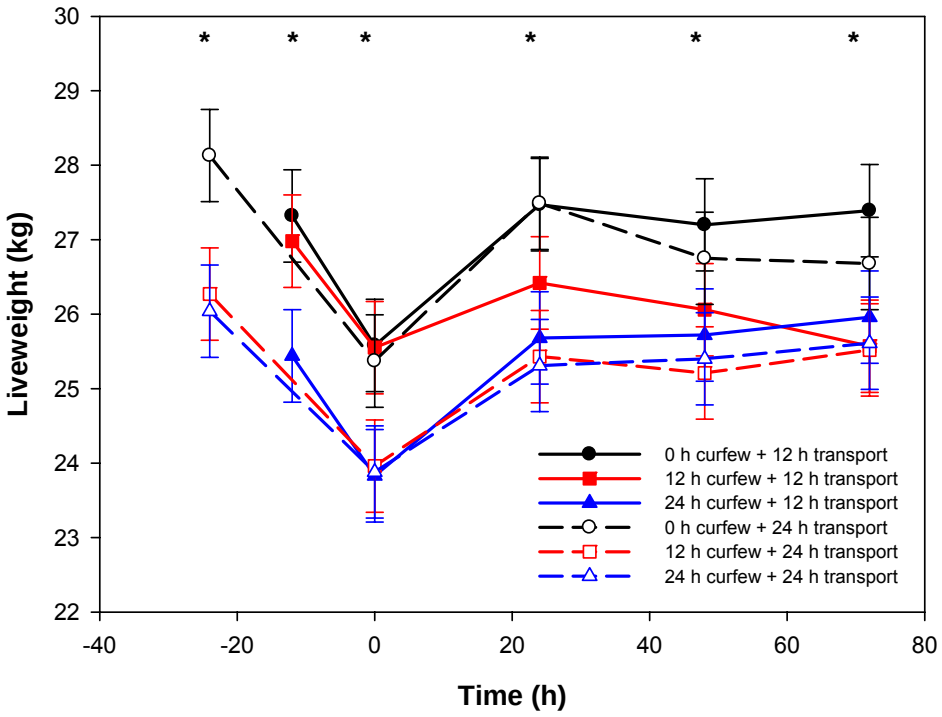


Figure 5 Least square means for liveweight for the interaction between curfew duration x transport duration x time. (* denotes a significant difference (P<0.05) between the curfew x transport treatment durations at each sampling time) Can you explain/comment on the different start weights for the 12hr curfew+12hr transp vs the 12hr curfew+24hrtransp given same curfew time?

Significant differences between the different curfew x transport treatments at 24, 48 and 72 h post-transport were evident. These were mainly due to the higher liveweights of the 0 h curfew durations. At the conclusion of the recovery period, the lambs had returned

to 91 – 98.5 % of their pre-transport mean liveweight. The lower figure pertains to the treatment combination of 12 h and 24 h curfew combined with 24 h transport.

(ii) Hydration measures

Only two of the indicators of hydration status (ALB and TP) were significantly influenced by the interaction of curfew treatment x transport treatment x time ($P<0.001$) (Tables 4 and 5). Moreover, the temporal changes over the transport and recovery phases were quite unexpected, particularly for TP (Figure 6d). Inexplicably, there was a reduction in TP and this was particularly evident for the groups transported for 12 h compared to the 24 h transport groups. Several of the contrasts between the individual treatment groups on arrival were significantly different ($P<0.05$). No further differences in TP between the treatment groups were evident during the remainder of the post-transport recovery phase.

Significant differences between the curfew/transport groups were also evident for serum ALB (Table 4, Figure 6e). However, the transport-mediated trends were less consistent compared to TP. Transport elicited an increase in only three of the six groups (0 h curfew + 12 h transport, 0 h curfew + 24 h transport and 12 h curfew + 24 h transport). Treatment differences (0 h curfew + 24 h transport < 24 h curfew + 12 h transport, $P<0.05$) were still evident after 24 h of recovery but not for the remainder of the recovery phase.

Serum osmolality which is the most informative dehydration measure was not affected by the interaction curfew duration x transport duration x time (Table 4). Nevertheless, the least square means are shown for this interaction in Figure 6c to illustrate that the normally expected haemoconcentration in response to water deprivation was still apparent. Notwithstanding the lack of significance, there was a clear increase in osmolality due to transport and the increase reflected the total time of water deprivation due to the curfew + transport durations. Significant differences in serum osmolality were only evident for the main effect of time ($P<0.001$) and the interactions of transport duration x replicate and curfew duration x replicate x time ($P<0.01$).

(iii) Cortisol

The interaction between curfew duration x transport duration x time was significant ($P<0.01$) for serum cortisol concentration (Table 4, Figure 6f). Relative to the pre-transport levels, the cortisol concentrations on arrival were higher for all treatment groups with the exception of the 24 h curfew + 12 h transport group. On arrival, the cortisol levels in the lambs from the 24 h curfew + 24 h transport group were significantly higher than the levels found in the 0 h curfew + 12 h transport, 24 h curfew + 12 h transport and the 0 h curfew + 24 h transport groups. The cortisol levels 24 h after transport were similar to the 0 h post-transport levels but there was a gradual decline for the remaining 48 h of recovery. The main exception here was the 0 h curfew + 12 h transport group.

(iv) Haematology

The temporal changes in the overall (WBC) and differential white blood cell (NEU and LYM and NEU:LYM) counts significantly differed between the curfew treatment x transport treatment groups (Table 5, Figure 6a&b). The WBC counts tended to be

higher at the conclusion of transport with the exception of the 12 h curfew + 24 h transport group (Figure 6a). This transport-mediated increase was much more consistent for NEU:LYM (Figure 6b). On arrival, the NEU:LYM for the 0 and 12 h curfew + 12 h transport groups was significantly greater than the 12 and 24 h curfew + 24 h transport groups.

A significant four-way ($P<0.01$) interaction between curfew duration x transport duration x replicate x time was observed for RCC. During the post-transport recovery phase, RCC significantly declined (Table 5) and the temporal changes differed between the curfew duration treatments through a significant interaction between curfew duration x time ($P<0.001$).

(v) Creatine kinase

Serum CK was significantly influenced by the three-way interactions between transport duration x replicate x time ($P<0.01$), curfew duration x replicate x time ($P<0.001$) and curfew duration x transport duration x replicate ($P<0.05$) (Table 4). Focusing on the last of these interactions and for simplicity, the interaction between curfew duration x transport duration is shown in Figure 7. This interaction was largely due to the treatment combination of 12 h curfew + 24 h transport where the CK levels were significantly higher than all the other curfew x transport duration groups. The only other significant treatment contrast was between the 0 h curfew + 12 h transport and 24 h curfew + 12 h transport.

From Table 4, it can also be seen that the CK levels declined during the post-transport recovery phase.

(vi) Blood urea nitrogen (BUN) and β-hydroxy butyrate (BHB)

The interaction curfew duration x transport duration x time was significant for BUN ($P<0.001$) and BHB ($P<0.05$) (Table 4, Figure 8b&c). The BUN levels prior to transport were positively associated with the duration of curfew. For the 12 h journey, the curfew mediated differences in BUN were significant. For the 24 h transport group, the BUN levels were significantly lower in the non-curfewed lambs compared to the curfewed lambs (12 and 24 h). With the exception of the 24 h curfew h +12 or 24 h transport groups, the serum concentrations of BUN increased in response to transport. Relative to the other treatment groups, the BUN levels on arrival were significantly lower for the 0 h curfew + 12 h transport group. The levels on arrival were not reflective of the total cumulative time of feed deprivation. Significant differences between the curfew x transport groups were also evident throughout the recovery phase. For BHB, there was a pronounced increase due to transport. The immediate post-transport BHB levels for the 0 h curfew + 12 h transport group were significantly lower than the 0 h or 24 h curfew + 24 h transport groups. Treatment differences during recovery were evident but this was largely due to the unusual increase in BHB levels in the lambs from the 0 h curfew + 12 h transport group. With the exception of this treatment group, there was a sharp decline in BHB levels during the initial 24 h of post-transport recovery.

(vii) Haptoglobin

Several significant interactions were found for the acute phase protein HAPT (Table 5). HAPT was not influenced by the interaction curfew duration x transport duration x time.

Curfew duration and transport (but not duration) elicited an increase in HAPT and these were gradually attenuated over the post-transport recovery phase.

Table 5 Least square means ± sem for curfew treatment, transport duration replicate and time and significance of the interactions for a subset of blood haematology measures (red cell count – RCC, haematocrit – HCT, white blood cell count – WCC, neutrophils – NEU, lympocytes – LYM, neutrophils:lymphocytes ratio – NEU:LYM), liveweight (LWT) and faecal score.

Main Effects	**RCC** ($x10^{12}$/L)	**HCT** (%)	**WCC*** ($x10^{9}$/L)	**NEU*** ($x10^{9}$/L)	**LYM*** ($x10^{9}$/L)	**NEU:LYM***	**HAPT*** mg/dL	**LWT** (kg)	**Faecal Score** (1-5)
Curfew duration (C)#									
0 h	10.50	34.19	6.75^{ab}	3.01^{ab}	2.33	1.29	0.15^{a}	26.94^{a}	2.41
12 h	10.35	33.45	7.05^{a}	3.38^{a}	2.28	1.48	0.16^{ab}	25.70^{b}	2.37
24 h	10.48	33.67	6.09^{b}	2.66^{b}	2.16	1.23	0.18^{b}	25.23^{b}	2.42
sem	0.18	0.40	-	-	-	-	-	0.43	0.07
Significance	*ns*	*ns*	*P<0.05*	*P<0.05*	*ns*	*ns*	*P<0.01*	*P<0.05*	*ns*
Transport duration (T)									
12 h	10.42	33.60	7.09	3.44	2.25	1.53	0.16	26.14	2.33
24 h	10.47	33.94	6.18	2.62	2.26	1.16	0.16	25.80	2.46
sem	0.15	0.32	-	-	-	-	-	0.35	0.05
Significance	*ns*	*ns*	*P<0.01*	*P<0.001*	*ns*	*P<0.001*	*ns*	*ns*	*ns*
Replicate (R)									
1	10.20	33.45	6.62	2.95	2.30	1.28	0.16	25.76	2.52
2	10.69	34.10	6.61	3.06	2.21	1.38	0.16	26.19	2.27
sem	0.15	*0.32*	-	-	-	-	-	0.35	0.05
Significance	*P<0.05*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*P<0.01*
Time#									
Pre-transport	10.92	35.33	6.16^{b}	2.84^{a}	2.02	1.41	0.15^{b}	26.70	2.20
0 h post-transport	10.82	34.87	6.97^{a}	4.08	1.72	2.38	0.20^{a}	24.69	1.56
24 h post-transport	10.37	33.66	7.08^{a}	3.19	2.59^{a}	1.23	0.18^{a}	26.30	2.89^{a}
48 h post-transport	10.16	32.91	6.37^{bc}	2.46	2.55^{a}	0.97^{a}	0.15^{b}	26.06^{a}	2.80^{a}
72 h post-transport	9.95	32.09	6.54^{c}	2.68^{a}	2.56^{a}	1.05^{a}	0.14	26.12^{a}	2.54
sem	0.11	0.25	-	-	-	-	-	0.25	0.06
Significance	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.001*
Interactions									
C x T	*P<0.05*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*P=0.06*
C x R	*P<0.05*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*P<0.01*	*P<0.05*
C x Time	*P<0.001*	*P<0.001*	*P<0.05*	*P<0.001*	*P<0.001*	*P<0.001*	*ns*	*P<0.001*	*P<0.001*
T x R	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*P<0.05*	*ns*
T x Time	*ns*	*ns*	*P<0.01*	*P<0.001*	*ns*	*P<0.001*	*P<0.05*	*P<0.001*	*P=0.05*
R x Time	*ns*	*ns*	*P<0.001*	*P<0.001*	*P<0.05*	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.05*
C x T x R	*ns*	*ns*	*ns*	*ns*	*P<0.01*	*ns*	*P<0.05*	*P<0.01*	*ns*
C x T x Time	*ns*	*ns*	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.001*	*ns*	*P<0.001*	*P<0.001*
C x R x Time	*ns*	*P<0.01*	*ns*	*P<0.01*	*ns*	*ns*	*P<0.05*	*P<0.001*	*P<0.01*
T x R x Time	*ns*	*P<0.01*	*P<0.05*	*ns*	*ns*	*ns*	*ns*	*P<0.001*	*ns*
C x T x R x Time	*P<0.01*	*P<0.05*	*ns*	*ns*	*ns*	*ns*	*ns*	*P<0.001*	*P<0.05*

*backtransformed least square means shown

Means without common superscripts are significantly different (P<0.05)

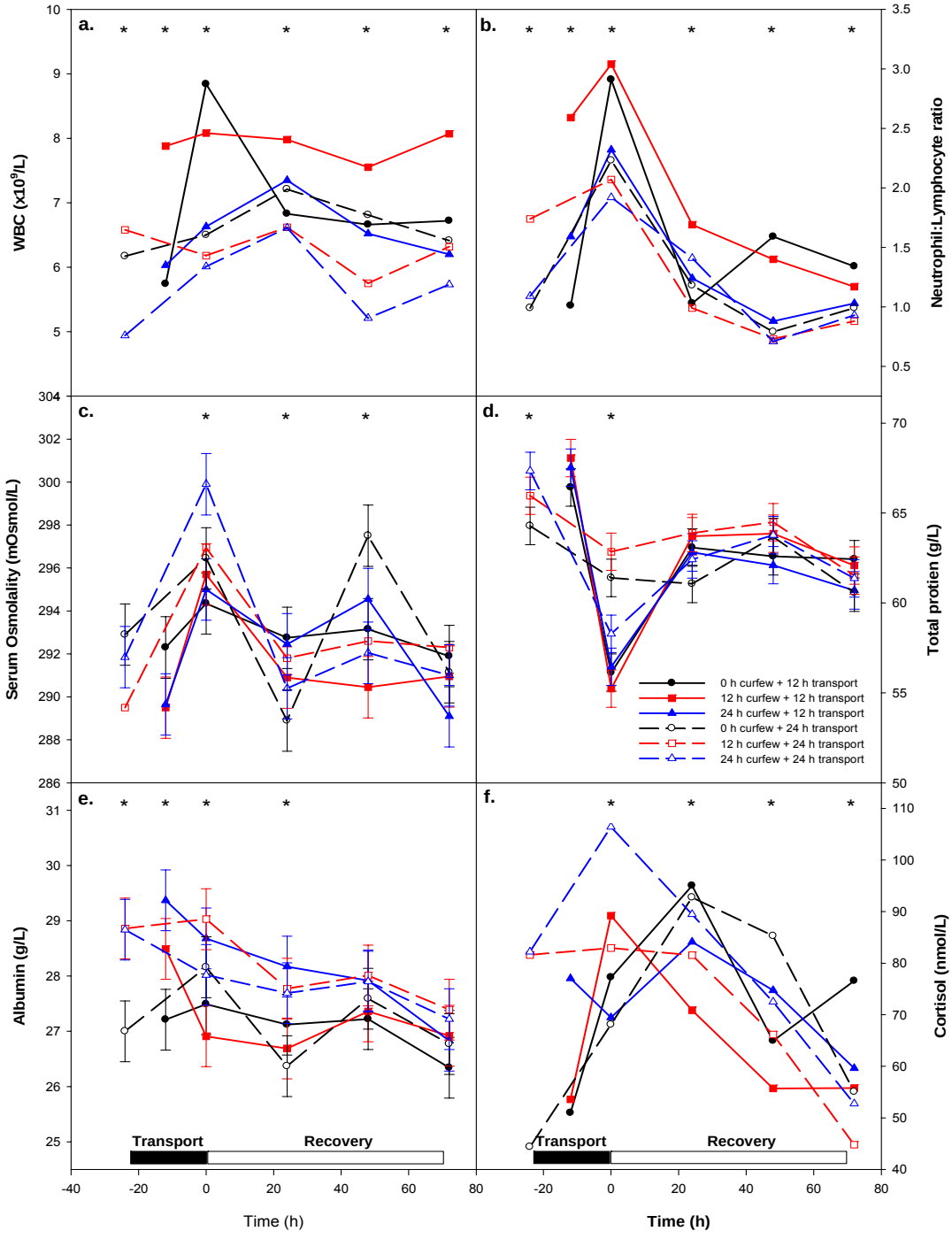


Figure 6 Least square means for a. white blood cell count (WCC), b. neutrophil:lymphocyte ratio, c. osmolality and the concentrations of d. total protein, e. albumin and f. cortisol for the interaction between curfew duration x transport duration x

time. (* denotes a significant difference (P<0.05) between the curfew x transport treatment durations at each sampling time)

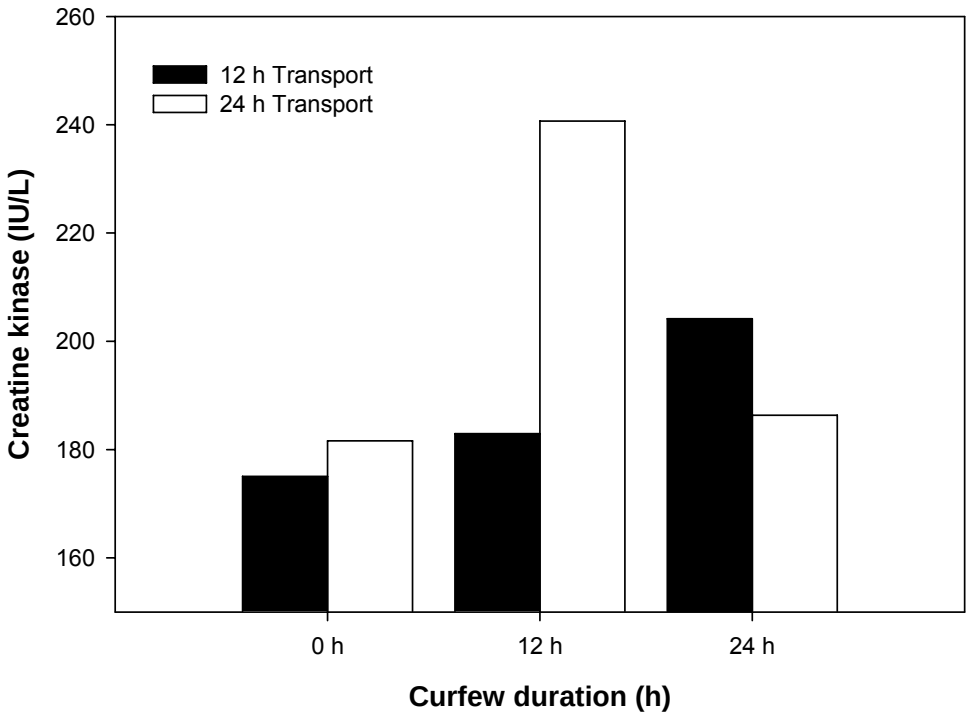


Figure 7 Least square means for serum creatine kinase concentration for the interaction between curfew duration x transport duration.

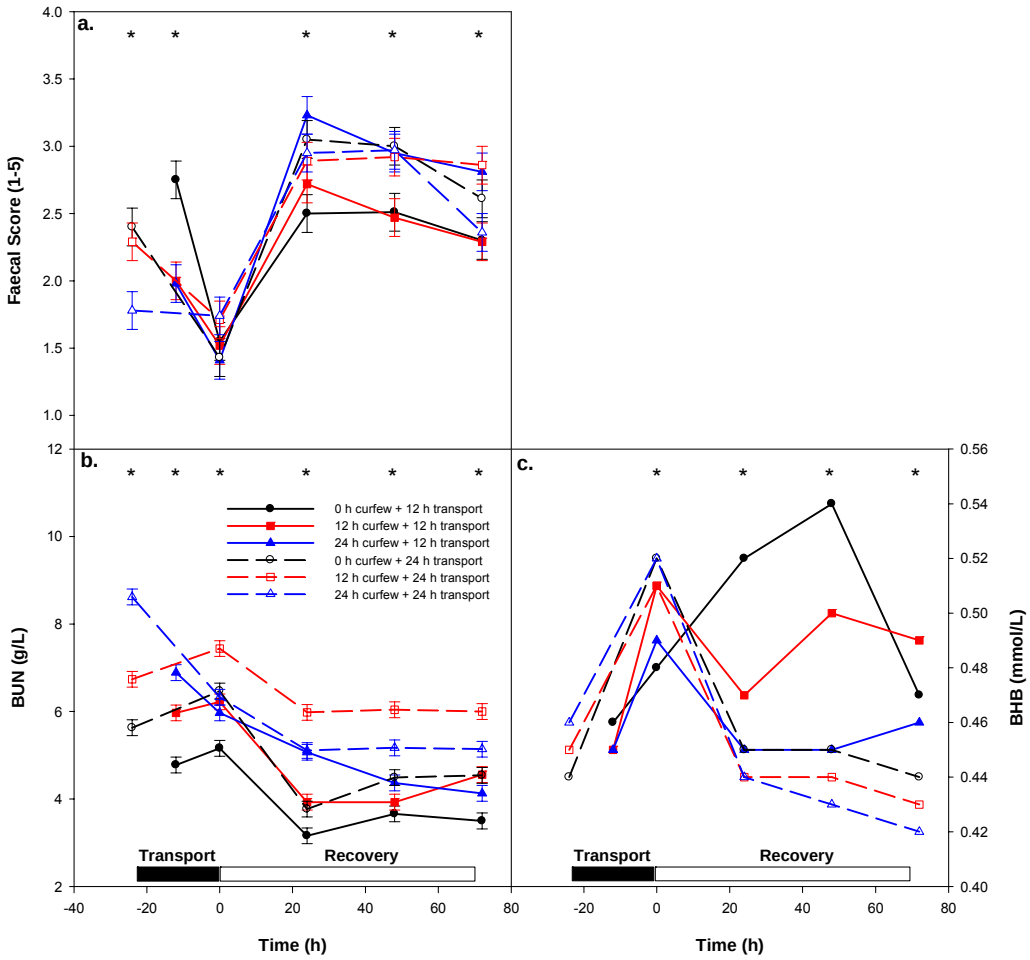


Figure 8 Least square means for a. faecal score, b. blood urea nitrogen (BUN), and c. β-hydroxybutyrate (BHB) for the interaction between curfew duration x transport duration x time. (* denotes a significant difference (P<0.05) between the curfew x transport treatment durations at each sampling time)

(vii) Fleece dirtiness scores and faecal scores

The fleece dirtiness score data was not amenable for statistical analysis because of the non-normality of the data. A summary of the change in the mean dirtiness scores after transport over the eight body regions is presented in Table 6. The body regions where the highest scores (ie. most dirty) were found after transport were the upper (including flank and brisket) and lower legs. The two non-curfewed groups had the highest overall mean difference in these regions.

Table 6 Change in the mean fleece dirtiness scores following transport

Curfew + Transport Duration	Body Regions							
	Rump & tail	Flank and upper hind leg	Lower hind leg	Midback	Brisket & upper fore leg	Lower fore leg	Neck	Head
0 h + 12 h	0.1	0.4	0.6	0.0	0.3	0.9	0.1	0.0
12 h + 12 h	0.2	0.3	0.0	0.1	0.2	0.0	0.0	0.0
24 h + 12 h	0.0	0.3	0.1	0.0	0.0	0.2	0.0	0.1
0 h + 24 h	0.0	0.8	1.0	0.0	0.6	1.0	0.0	0.0
12 h + 24 h	0.2	0.1	0.2	0.0	0.2	0.1	0.0	0.0
24 h + 24 h	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

The analysis of faecal scores revealed a significant interaction between curfew duration x transport duration x time (Table 5, Figure 8a). For the 12 h transport duration, the pre-transport faecal scores were significantly higher (ie. higher in moisture) for the 0 h curfew duration compared to either 12 or 24 h curfew treatments. In the case of the 24 h transport duration, a similar trend was observed where the pre-transport faecal scores for the 0 and 12 h curfew groups were significantly higher that the 24 h curfew group. Transport resulted in a large decline in faecal score but there were no differences between the two transport durations on arrival. The faecal scores increased after 24 h of recovery and remained relatively constant for the remainder of the recovery phase. Treatment differences were still evident during the recovery phase and this was largely due to the 0 and 12 h curfew + 12 h transport groups which had lower scores than some (at 24 and 72 h post-transport) or all (48 h post-transport) of the other treatment groups.

Images of the excreta levels at the conclusion of the transport phase are shown in Appendix 3. Based on subjective observations, the excreta appeared to be more fibrous and drier as the total time of feed and water deprivation increased. The moisture content of the excreta for the 2nd replicate of the 12 h curfew + 12 h transport treatment was quite high but this was largely due to a rainfall event during transport.

3.3 Effect of curfew and transport duration on the shedding of enteric pathogens

The complete report prepared by Food Science Australia is attached as Appendix 4. The key conclusion was that there were no significant effects of curfew or transport duration on the levels of *E.coli* in the faeces from the lambs.

3.4 Effect of curfew and transport duration on lying behaviour during and after transport

(i) During the journey

The results of the analysis of the time spent lying during transport are shown in Table 7. Only two of the lambs did not lie down during the journey. Therefore it was amenable for statistical analysis using the GLM procedure in SAS after normalising the data.

Table 7 Least square means ± sem for curfew treatment, transport duration and replicate and significance of the interactions for lying time during transport

Main Effects	Lying time during transport (min)
Curfew duration (C)	
0 h	4.00
12 h	7.19
24 h	6.73
sem	-
Significance	*ns*
Transport duration (T)	
12 h	3.66
24 h	9.14
sem	-
Significance	*P<0.01*
Replicate (R)	
1	7.25
2	4.62
sem	-
Significance	*ns*
Interactions	
C x T	*P<0.01*
C x R	*ns*
T x R	*ns*
C x T x R	*ns*

A significant interaction between curfew duration x transport duration was found (Table 7) and this is shown in Figure 9. The mean lying time for the different curfew x transport duration treatments ranged from 2 – 20 min. Lambs spent significantly more time lying during transport in the 12 h curfew + 24 h transport and 24 h curfew + 24 h transport groups compared to all other treatment combinations. The only exception here was the contrast between the 0 h curfew + 12 h transport and 24 h curfew + 24 h transport where the difference was not significant. Over the shorter transport duration of 12 h there was no difference in the lying time between the different curfew duration treatments.

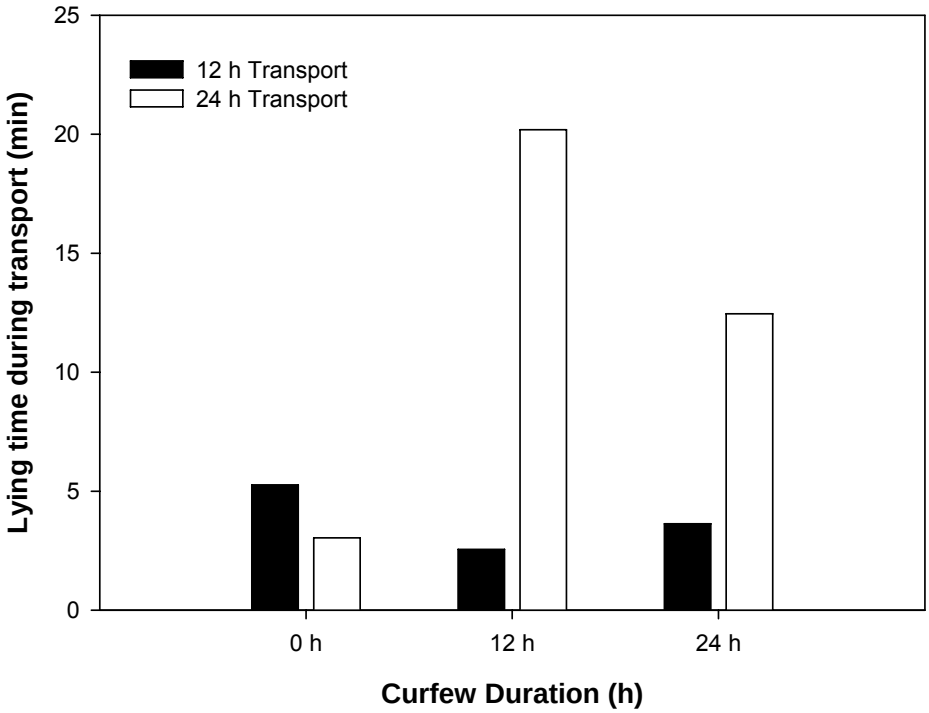


Figure 9 Least square means for lying time during transport (min) for the interaction between curfew duration x transport duration.

(ii) During initial 24 h of recovery

In the repeated measures analysis of lying behaviour during the initial 24 h of recovery, a significant interaction ($P<0.001$) between curfew duration x transport duration x recovery period (initial 24 h) was found (Figure 10a). During the initial 3 h of recovery, there was no difference in the lying time between any of the curfew x transport duration groups. Significant differences were observed between the treatment groups at each 3 h period over the initial 24 h however, there was no consistent trend. The exception being the 12 h curfew + 12 h transport group which spent less time lying over first 24 h of recovery. Lying time tended to increase over the 24 h for most treatment groups and the groups transported for 24 h tended to spend more time lying during the last 12 h of initial recovery.

(iii) During 72 h recovery

Over the full 72 h of recovery, the interaction between curfew duration x transport duration x recovery period (3 x 24 h) was significant ($P<0.01$) (Figure 10b). During the initial 24 h period, the 0 and 12 h curfew + 12 h transport groups spent significantly less time lying than the other treatment groups ($P<0.05$). This trend was still evident in the second 24 h recovery period particularly for the 12 h curfew + 12 h transport group. There was a trend for groups transported for 24 h, particularly the 0 and 12 h curfew treatment groups, to spend more time lying during the second and third recovery days. There was a general increase in lying time for most groups during the 72 h of recovery.

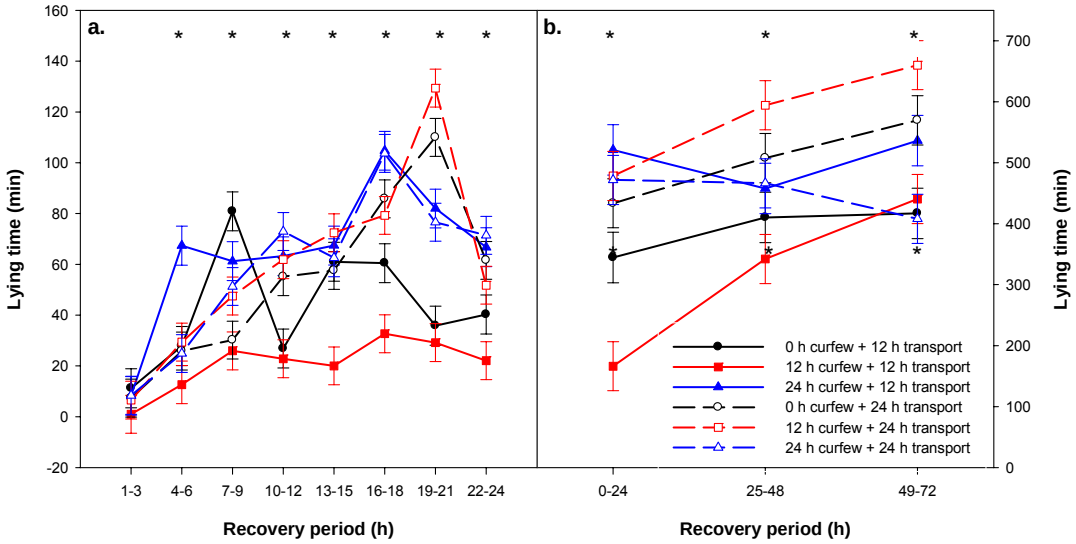


Figure 10 Least square means for lying time over the a. initial 24 h (8 x 3 h periods) and b. 72 h (3 x 24 h periods) of post-transport recovery for the interaction between curfew duration x transport duration x recovery period. (* denotes a significant difference (P<0.05) between the curfew x transport treatment durations at each sampling time)

3.5 Effect of curfew and transport duration on water consumption during 72 h of post-transport recovery

Water consumption during recovery was not affected by any of the main experimental effects or interactions. Lambs that were subjected to 24 - 48 h of water deprivation through the combined effects of curfew and transport consumed more water during the initial 24 h of recovery compared to the group subjected to only 12 h of water deprivation (ie. 0 h curfew + 12 h transport) but the differences were not significant (Table 8). The results presented in Table 7 also illustrate quite clearly that the daily consumption levels were very low.

Table 8 Mean water consumption (L) of lambs in response to combined periods of water deprivation (curfew + transport duration)

Total time of water deprivation (curfew + transport)	Recovery Period (h)		
	1 - 24	25 - 48	49 - 72
12 h	1.0	0.5	0.0
24 h	5.4	0.9	4.4

36 h	5.4	4.0	4.0
48 h	5.0	0.25	1.75

4. Discussion

The results of this investigation generally indicate that subjecting healthy, grass-fed lambs to pre-transport periods of food and water deprivation (12 and 24 h compared with 0 h controls) before transport for 12 or 24 h did not adversely affect animal welfare. The response indicated that the overall feed and water deprivation period and its associated effects was additive across the curfew and transport periods. Furthermore, subjecting the lambs to pre-transport periods of food and water deprivation (12 and 24 h compared with 0 h) prior to transport for 12 or 24 h did not appear to enhance the capacity of the animals to cope with transport.

From an animal welfare perspective, it is the physiological states of fatigue and dehydration that are of most concern in the context of livestock transport particularly over moderate to long durations (12 – 48 h). With regard to dehydration, the results based on some of the serum measures of haemoconcentration were quite unexpected. For example, with increasing periods of water deprivation, the total protein (TP) concentration in blood typically increases (Knowles and Warriss 2000). However, in the current experiment there was a minimal effect of curfew duration on serum TP and surprisingly the levels actually declined in response to transport. The latter does not appear to be underpinned by the associated changes in serum ALB levels so it would appear that there has been a marked reduction in serum globulin levels. There are no obvious reasons why this should have occurred and it is difficult to explain.

Other measures such as haematocrit (HCT) and serum osmolality (OSMOL) were also not significantly affected by the main effects of curfew and transport duration or by key interactions such as curfew duration x transport duration x time. Although not significant, the temporal changes in OSMOL in response to curfew and transport does however, reflect some haemoconcentration and this was generally proportional to the total time of water deprivation. Whilst we are not trying to emphasise a non significant result, the latter does lend further support to the value of OSMOL as a more sensitive and informative marker of dehydration.

The enforced water deprivation in the present study did not have any significant impact on the hydration status of the animals which supports the results of Parrot et al (1998). In their study, 50 kg lambs were transported for 31 h with a 1 h rest stop after 14 h where they were unloaded and had access to feed and water. In that study, the serum OSMOL was lower at the conclusion of the journey compared to the levels measured pre-transport. Very few animals drank during the rest period and these authors concluded that dehydration was not likely to be an issue in sheep during journeys of 31 h.

The water consumption patterns, particularly during the initial 24 h of recovery, also did not indicate that lambs subjected to longer periods of water deprivation were more

dehydrated. However, it must be stressed that the water consumption levels throughout the 72 h post-transport recovery period were remarkably low. The lambs had prior experience with the watering facilities in the plots so the reduced consumption levels were unlikely to be due to any lack of familiarity with them. Rather, the low water intakes were more likely to be due to the moderately high moisture content of the pasture (50 – 60%) and high overnight dew levels encountered during the experiment. Given this, some caution needs to be applied in the interpretation of the post-transport water consumption results.

There are a small number of investigations comparing the physiological effects of similar periods of food and water deprivation or transport in both cattle and sheep (Gaylean et al 1981, Knowles et al 1995, Parker et al 2003a). The evidence from these studies suggests that the differences in the physiological responses to similar periods of food and water deprivation achieved through a conventional curfew or transport are negligible. These results seem counterintuitive given the additional psychological stress and physical demands that occur during transport. Psychological stress can induce diuresis (Parker et al 2003b) and therefore, it is reasonable to expect that fluid losses might be higher during transport. The psychological stress associated with transport, based on changes in heart rate and plasma cortisol concentrations, was generally highest during loading and initial phases of transport (Eldridge et al 1988, Warriss et al 1995, Pettiford et al unpublished). Beyond that, animals generally habituate to the transport conditions. Consequently, the elevated stress response is generally not sustained over the entire journey. The effort to maintain balance during transport would also be expected to incur increased muscular demands compared to that during food and water deprivation only. However, Knowles et al (1995) reported no difference in plasma creatine kinase levels (an indicator of muscle fatigue and use) in sheep transported for 24 h versus those deprived of food and water for 24 h.

The behaviour, specifically lying time, during and subsequent to transport and serum creatine kinase levels provide insights into the level of fatigue experienced by the lambs. The serum creatine kinase (CK) results did not reveal any major treatment differences. The changes in CK due to transport duration were not large or consistent between the 12 and 24 h transport treatments. Increased lying during transport was more apparent during the longer 24 h journey and the incidence of lying behaviour was reasonably even over the 24 h of transport. For example, the mean (± sd) lying time during the first, second and third 8 h of travel were 23.7 ± 46.3, 20.1 ± 29.2 and 24.9 ± 42.8 min, respectively. The even spread of lying time over the journey perhaps indicates that fatigue may not necessarily have been the primary driver for this behaviour. This coupled with the lack of any consistent transport duration differences in CK levels, suggests that fatigue *per se* may not have been a major factor in this study. In a study by Knowles et al. (1995) where 38-kg sheep were transported by truck for 3, 9, 15, 18 or 24 hours and given sufficient space for the animals to lie down (0.29 m^2 per animal), the animals remained standing for the first 4 hours, but then the majority lay down for the remainder of the journeys unless disturbed.

Another salient result in this study was that all but two animals spent some time lying during the journey. This is in contrast with the results observed in the sheep transport duration experiment (refer AHW.055 Milestone 8 Report). In that experiment, only 18

out of 120 experimental sheep were observed to lie down during journeys of 12, 30 or 48 h. Although there were differences in the vehicles used in the two experiments, the other major difference was the age of the animals (lambs versus 4-5 year old ewes). It is possible that younger animals were less concerned about the consequences of lying during transport.

An important finding of the present study was the significant increase in lying behaviour in the curfewed compared to non-curfewed lambs transported for 24 h. This was also apparent in the cattle curfew x transport duration experiment undertaken as part of this project (refer AHW.055 Milestone 6 Report). Collectively, these results do not align with the anecdotal views and observations from livestock transporters that non-curfewed cattle and sheep are more likely to go down during transit, particularly in the earlier stages of a journey (i.e. before they have had a chance to 'empty out'). It is also worth noting that it was not possible to distinguish whether the lying behaviour was preceded by a slippage event and loss of balance. Slippage events could increase in non-curfewed animals due to the increased volume of excreta however, we can only speculate whether this also leads to an increased risk of animals going down.

The differences in lying behaviour during the recovery phase does not conclusively indicate that the animals transported over longer durations were more tired than the animals transported over the shorter duration of 12 h. This contrasts with the observations by Ferguson et al (2006) in cattle. It is likely the greater tolerance of sheep to stand for long periods during transport can be attributed to their lighter mass compared to cattle.

Of the serum measures of metabolic change, BUN was more consistently affected by curfew duration rather than transport duration or the combined effects of curfew and transport. Transport rather than curfew duration on the other hand, was more influential on the serum changes in BHB and there wasn't a consistent trend with regard to the combination of curfew and transport. This result tends to suggest that the increase in BHB after transport may have been mediated more by sympatho-adrenal activation rather than total period of feed deprivation. Overall, the serum concentrations of BHB were generally higher in these lambs compared to the results in other sheep transport studies (Knowles et al 1995, AHW.055 Milestone 8 Report) but the levels were still within the normal range.

The trends in the rectal temperature profiles align with the general view that loading and the initial stages of transport are the most stressful (Eldridge et al 1988, Warriss et al 1995, Pettiford et al 2007). After 3-4 h of transport, rectal temperatures returned and remained at normal levels during the journey and then increased again during unloading. The effects of the different curfew and transport durations on serum cortisol concentration were generally inconsistent. The immediate post-transport cortisol levels were higher than the pre-transport levels but not in all groups (eg. 24 h curfew + 12 h transport). The general decline in the cortisol levels over the 72 h post-transport recovery phase may reflect some habituation by the lambs to the process of being handled and blood sampled.

Transport caused an increase in the total white cell (WBC) and neutrophil counts and neutrophil:lymphocyte ratio (NEU:LYM) which is consistent with the findings observed previously in this project. This increase was particularly evident for the 0 and 12 h curfew groups transported for 12 h. The lower WBC and NEU:LYM response in the 24 h transport groups on arrival was probably due to the fact that there had been some attenuation of response over the longer journey duration.

The effects of the total time of feed and water deprivation (ie curfew + transport) on liveweight lost was consistent with other published data. Wythes and Morris (1994) reviewed several experiments where liveweight was measured in sheep over different periods of food and/or water deprivation. They reported a mean weight loss after 12 and 48 h of 5% and 11 %, respectively, which is similar to the results (4.6 % - 12 h and 7.8 % - 48 h) in the present study. The rate of liveweight recovery during the 72 h post-transport recovery phase was quite rapid during the initial 24 h but then tended to plateau. After 72 h of recovery, the transport groups had reached between 91.0 – 98.5% of their pre-transport liveweights.

Pre-transport curfews resulted in a decrease in subjective faecal scores indicating a reduction in moisture content in the faeces. Transport resulted in a further reduction in faecal score but not necessarily in any duration dependent manner. Lambs not subjected to any pre-transport curfew tended to have dirtier fleeces particular in the upper and lower legs at the conclusion of transport. Collectively these results and those of others (Gregory et al 2000) lend support for the application of pre-transport curfews to reduce the moisture level and total amount of faeces and therefore level of faecal contamination on animals. For sheep destined for slaughter, this is clearly beneficial from a food safety perspective. However, whilst curfews reduce the risk of faecal soiling on animals, there is evidence that indicates they may also result in increased shedding of enteric pathogens such as *E.coli* O157 and *Salmonella* (Pointon et al 2006). The faecal samples collected as part of the current study were analysed for the presence of *E. coli* (including *E. coli O157*) and *Salmonella*. There was no effect of either curfew or transport duration on total E.coli counts. Moreover, *E. coli O157* and *Salmonella* were not detected in the faeces. This suggests that applying these feed and water withdrawal and transport times may have little impact on the presence of E. coli and Salmonella in sheep faeces, and subsequently the risk of contamination of carcases. If microbes didn't change but the level of soiling was reduced in curfewed animals why would there not be support for curfew to reduce fleece soiling?

The evidence presented here does not lend support to the view that pre-transport curfews facilitate improvements in the capacity of sheep to cope with transport. If animals go down during transport then there is an increased risk of injury and death through trampling and smothering, respectively. Clearly, under these circumstances animal welfare is compromised. The evidence presented here suggests that even though the curfewed animals tended to lie down more in transit, the period of lying was not long and they had the strength to return to their feet. Therefore, in this instance, the risk that animal welfare could be negatively affected was relatively low. The condition and physiological state of the stock prior to transport is likely to be a key factor on the animal welfare outcomes when animals go down during transport.

Overall, the results generally indicate that the lambs in this study coped exceptionally well with periods of food and water deprivation up to 48 h due to the combined duration of curfew and transport. Pre-transport curfews (without water in particular) merely extend the total period of time the animals are deprived of water during the transport event. However there will be some production circumstances (eg. lambs coming off lush pasture) where a short pre-transport curfew (e.g. 6 h) may be desirable and this is unlikely to be deleterious on animal welfare grounds. Given 12 hr curfew was tested it would be good if you could provide some information to explain why 6 hrs is suggested here.

5. Conclusions

The results of this investigation indicate that pre-transport feed and water withdrawal (12 and 24 h compared with 0 h) prior to transport for 12 or 24 h did not adversely affect animal welfare, but simply added to the overall feed and water deprivation period and its associated effects. Therfore, subjecting healthy, grass-fed lambs to pre-transport periods of food and water deprivation did not appear to enhance the capacity of the animals to cope with transport. However, we can conclude that lambs in good physiological condition can cope with periods of up to 48 h of food and water deprivation without any major deleterious affect to their welfare.

6. References

Cockram, M. S., Baxter, E. M., Smith, L. A., Bell, S., Howard, C. M., Prescott, R. J. & Mitchell, M. A. 2004. Effect of Driver Behaviour, Driving Events and Road Type on the Stability and Resting Behaviour of Sheep in Transit. *Anim. Sci*. **79**: 165-176.

Eldridge, G.A., Winfield, C.G. and Cahill, D.J. (1988). Responses of cattle to different space allowances, pen sizes and road conditions during transport. *Aust. J. Exper. Agric.* **28**:155-159.

Galyean, ML, Lee, RW and Hubbert, ME (1981). Influence of fasting and transit on ruminal and blood metabolites in beef steers. *J. Anim. Sci*. **53**: 7-18.

Gregory, NG, Jacobsen, LH, Nagle, TA, Muirhead, RW and Leroux, GJ (2000). Effect of preslaughter feeding system on weight loss, gut bacteria and the physico-chemical properties of digesta in cattle. *NZ J. Agric. Res.* **43**: 351-361.

Jones, GE and Mould, DL (1984). Adaptation of the guaiacol (peroxidase) test for haptoglobins in a microtitration plate system. *Res. Vet. Sci*. **37**:87-92.

Kaneoko, JJ, Harvey, JW and Bruss, M.L. Eds. (1997). "Clinical Biochemistry of Domestic Animals" 5th Edition. (Academic Press, London, UK).

Knowles, T.G. and Wariss, P.D. (2000). Stress physiology of animals during transport. In: "Livestock Handling and Transport" 2nd Edition. Ed T.Grandin, (CAB International, Oxon UK).

Knowles, TG, Brown, SN, Warriss, PD, Phillips, AJ, Dolan, SK, Hunt, P, Ford, JE, Edwards, JE and Watkins, PE. (1995). Effects on sheep of transport by road for up to 24h. *Vet. Rec*. **136**: 421-438.

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

Parker, AJ, Hamlin, GP, Coleman, CJ and Fitzpatrick, LA (2003b). Dehydration in stress ruminants may be the result of cortisol-induced diuresis. J. Anim. Sci. 81: 512-519.

Parrott, R.F., Hall, S.J.G., Lloyd, D.M., Goode, J.A. and Broom, D.M. (1998). Effects of a Maximum Permissible Journey Time (31 H) on Physiological Responses of Fleeced and Shorn Sheep to Transport, With Observations on Behaviour During a Short (1 H) Rest-Stop. *Anim Sci.* **66**: 197-207.

Pettiford, S.G., Ferguson, D.M., Lea, J.M., Lee, C., Paull, D., Reed, M.T. and Fisher, A.D. (2006). Milestone Report - The effect of transport duration on indicators of cattle welfare. MLA Project AHW.055 – Animal welfare outcomes of livestock road transport practices.

Pointon, A, Kiermeier, A and Fegan N. (2006). Review of food safety and carcass hygiene. MLA Project LIVE122A Final Report - Investigating feed and water curfews for the transport of livestock within Australia - a literature review.

Slocombe, L.L. and Colditz, I.G. (2005). Evaluating the stress of production in cattle using haptoglobin. 5th Annual International Colloquium on Animal Acute Phase Proteins, Dublin, Ireland.

Tarrant, V. & Grandin, T. 2000. Cattle Transport. In: Grandin, T. (ed.) Livestock Handling and Transport., pp. 151-173. Cabi publishing, Wallingford, U.K.

Tarrant, P. V., Kenny, F. J., Harrington, D. & Murphy, M. 1992. Long Distance Transportation of Steers to Slaughter: Effect of Stocking Density on Physiology, Behaviour and Carcass Quality. *Livest. Prod. Sci.* **30**: 223-238.

Warriss, P.D., Brown, S.N., Knowles, T.G., Kestiin, S.C., Edwards, J.E., Dolan, S.K. and Phillips, A.J. (1995). Effects on cattle of transport by road for up to 15 hours. *The Vet. Rec.* **126**:319-323.

Overall progress of the project

The project is in its final stages. The next and final milestone is the submission of the final report which is on schedule for completion by the due date (5/9/07).

Recommendations

The practice of pre-transport curfews for lambs is highly variable in its application in terms of the duration of curfew and whether the sheep are deprived of feed or water or both. The generic application of some period of pre-transport curfew, irrespective of the transport duration and nutritional background of the lambs, is highly questionable in terms of benefits. The outcomes of this investigation indicate that subjecting healthy, grass-fed lambs to pre-transport periods of food and water deprivation (12 and 24 h compared with 0 h) prior to transport for 12 or 24 h did not enhance the capacity of the animals to cope with transport. Given this, it is recommended that the need for pre-transport curfews should be predicated on consideration of key factors such as the nutritional background and condition of the lambs and the duration of the transport.

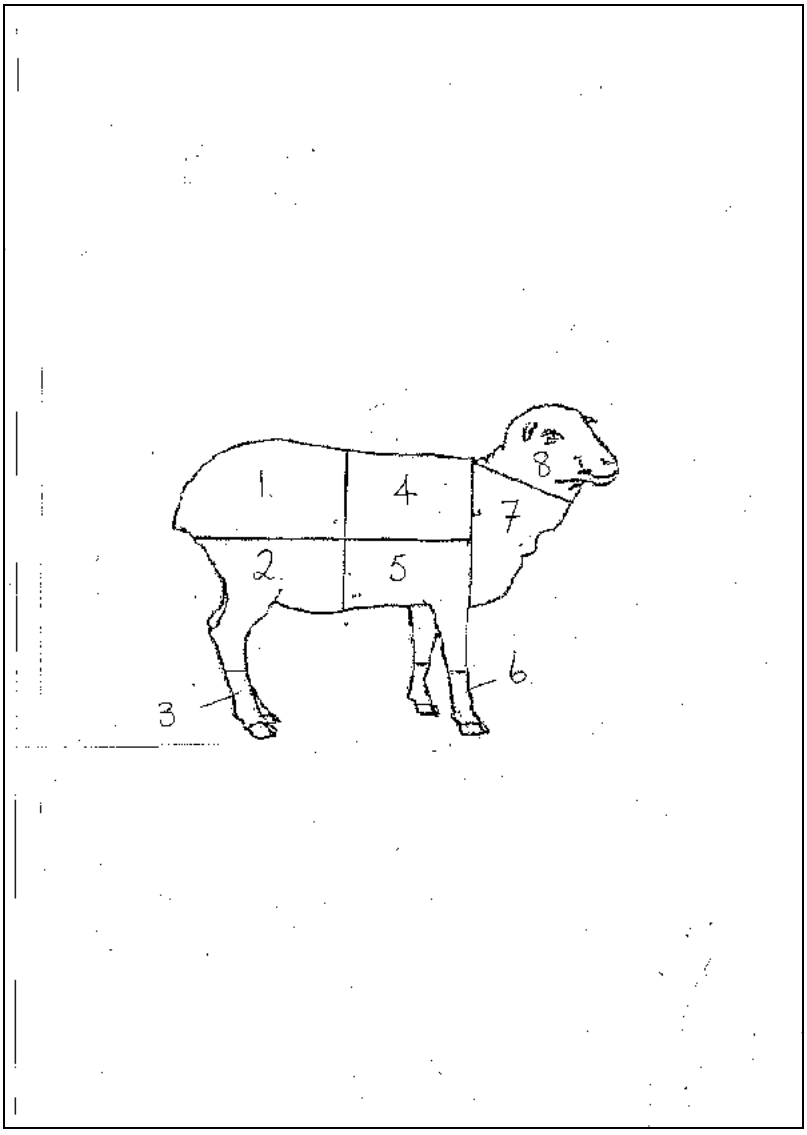
Appendix 1 Faecal consistency score

Faecal consistency score (FCS) was measured on a scale of 1 to 5. The description for each faecal consistency score are outlined below:

Faecal score	Description
1	hard pellet, dry to touch, “cracks” readily under firm pressure between finger and thumb
2	soft pellet, moist to touch, less tendency to “crack” under firm pressure between finger and thumb
3	soft faeces, loss of pellet structure, firm paste, tube-like appearance
4	pasty diarrhoea, soft paste
5	watery, liquid diarrhoea

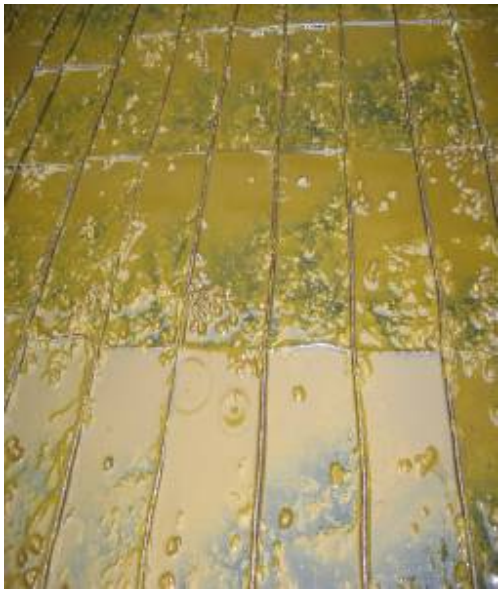
Appendix 2 Body regions and fleece dirtiness scores

Score 0 clean
Score 1 slightly dirty or soiled
Score 2 moderately dirty or soiled
Score 3 very dirty or soiled



Appendix 3 Post-transport excreta levels on truck floor.


0 curfew 12hr transport

12hr curfew 12hr transport


0 curfew 24hr transport


12 curfew 24hr transport

24hr curfew 12hr transport

24hr curfew 24hr transport

Appendix 4

Results for today Ideas for tomorrow

9.1.1.1.1.1.1 PROJECT A.MFS.0119

FOOD SCIENCE AUSTRALIA

A joint venture of CSIRO & the Victorian Government

Effect of curfew on the microbiology of sheep

a report prepared for Meat & Livestock Australia

mla MEAT & LIVESTOCK AUSTRALIA

July, 2007

Food Science Australia

Brisbane Laboratory
Cnr Wynnum and Creek Roads
Cannon Hill
Queensland 4170

9.2 Table of Contents

9.3 Executive Summary

The effect of 12 or 24h transport and food and water deprivation (FWD) for 12 and 24h prior to transport on the levels of *E. coli* in the faeces of sheep was investigated. The presence and numbers of *E. coli* O157 and *Salmonella* was also determined. Faecal samples were collected from each of 10 sheep before any treatment, after 12 or 24h FWD, after 12 or 24h transport and then after 24, 48 and 72h recovery after transport. A total of 680 faecal samples were collected and tested for the numbers of *E. coli* present and the presence and numbers of *E. coli* O157 and *Salmonella*.

E. coli was enumerated in 677 samples as the number of *E. coli* could not be determined for 3 samples (either because of overgrowth by coliforms or because the count was below the level of detection which was <10 cfu/g). The mean $\log_{10}$ count of *E. coli* in the faecal samples was 6.05 cfu/g with counts ranging between $\log_{10}$ 1.7 to 8.61 cfu/g. Mean $\log_{10}$ counts of *E. coli* in the faeces of groups of sheep varied between 4.47 and 7.6 cfu/g throughout the experiment. The mean $\log_{10}$ count of *E. coli* in faecal samples of sheep before treatment was 5.9 cfu/g, after 12h FWD was 6.05 cfu/g, after 24h FWD was 6.15 cfu/g, after 12h transport was 5.76 cfu/g, after 24h transport was 6.33 cfu/g, then after 24, 48 and 72h recovery was 6.35, 6.03 and 5.87 cfu/g respectively. There appeared to be no consistent trend observed for mean counts of *E. coli* in the faeces of sheep at different stages of the experiment. There were no significant interactions between the mean $\log_{10}$ counts of *E. coli* in the faeces of sheep regardless of FWD or transport times. It appears the counts of *E. coli* sometimes fluctuate after FWD or transport but not in a consistent way.

E. coli O157 and *Salmonella* were not detected in this study and no conclusions can be offered about the effect of FWD and transport on these pathogens. This does suggest if there were very low numbers present they were not amplified by these potentially stressful activities. Testing for Shiga toxin genes (*stx*) in the faeces of sheep was performed to ascertain if there was any effect of FWD and transport times on the presence of these genes. Shiga toxins are predominantly harboured by *E. coli* and even though the total numbers of *E. coli* may not have varied significantly between treatments, the type of genes the *E. coli* possess may have been affected. The faecal samples collected from sheep were tested for both types of *stx*, stx_1 and stx_2. There were no significant differences observed in the prevalence of these genes between different FWD and transport treatments. In general, there was a decline in the prevalence of *stx* in the faeces of sheep after FWD and transport, but this difference was not significant. No interactions between FWD or transport treatments were observed.

In conclusion, there appears to be no significant effect of 12 or 24h FWD or transport for 12 or 24h on the levels of *E. coli* found in the faeces of sheep. This suggests that applying these FWD and transport times will have little impact on the presence of *E. coli* and *Salmonella* in sheep faeces, and subsequently the risk of contamination of carcases.

9.4 Introduction

In order to protect public health and maintain consumer confidence and export markets for Australian meat, it is important to control pathogens through the food chain. This includes understanding the factors influencing the shedding of foodborne pathogens such as *Salmonella* and pathogenic strains of *Escherichia coli* by animals. There has been limited work investigating the impact of food and water deprivation (FWD) pre-slaughter on the shedding of pathogens by animals (Grau et al. 1969; Kudva et al. 1995). Sheep may become stressed by activities such as a change in diet (Kudva et al. 1995), food deprivation (Grau et al. 1969) and transport (Knowles 1998) which may lead to increased faecal pathogen shedding. Animals that shed higher numbers of pathogens at slaughter are more likely to be a source of cross contamination of other animals, the transport and lairage environments and the meat that is subsequently produced. It is therefore important for the industry to have information that can be used to assess the risks that transport and feed and water withdrawal times contribute to the microbiological quality of meat.

Project AHW.055 was set up to investigate the effect of pre-transport FWD on the behavioural and physiological responses resulting from transporting young sheep. This provided an opportunity to gather samples from sheep to test for effects of FWD and transport duration on the shedding of food borne pathogens. The numbers of *E. coli* and the presence of *Salmonella* on sheep carcases form part of the national *E. coli* and *Salmonella* monitoring program (ESAM). *E. coli* O157 is also an important pathogen with respect to the export beef industry. The aim of this study was to test faecal samples collected from the sheep enrolled in project AHW.055 for the numbers of *E. coli* and the levels and prevalence of *E. coli* O157 and *Salmonella* in their faeces. This study will determine if particular FWD and transport times lead to an increase or decrease in these microorganisms. FWD and transport times which lead to high counts of *E. coli* and pathogens may compromise the safety of meat produced from such animals and should be avoided. This study will provide information that can be used to guide the industry in providing curfew guidelines that will meet food safety requirements and animal health when combined with AWH.055.

9.5 Methods

9.5.1 Samples

Samples were collected as part of project AHW.055. A factorial design was applied to the collection of samples from sheep, which comprised 2 replicates of 3 pre-transport

FWD treatments (0, 12 and 24 h of feed and water deprivation) and 2 transport duration treatments (12 and 24 h). A total of 12 groups of sheep were sampled with 10 sheep from each group sampled after each treatment (Table 1). Rectal faecal samples were collected by project staff in AHW.055 at the designated time points corresponding to other data collection (eg. blood samples etc). A fresh glove was used to collect faeces from each animal, which were placed into a specimen jar and stored chilled until processed. When faeces were not present in the rectum of an animal, a cotton tip swab was used to collect material from the rectal area. Swabs were placed in sterile 10ml containers and stored with other faecal samples. Samples were transported under chilled conditions via overnight courier to the Food Science Australia, Cannon Hill laboratories. The maximum time samples were stored in the chiller before processing was 5 days. Storage for this length of time has been shown to have no effect on the numbers of *E. coli* present in sheep faeces (data not shown).

Table 1. Treatment of different groups of sheep and points of faecal collection

	Animal group number											
Treatment	1	7	4	10	2	8	5	11	3	9	6	12
Before treatment	+[a]	+	+	+	+	+	+	+	+	+	+	+
12h FWD					+	+	+	+				
24h FWD									+	+	+	+
12h Transport	+	+			+	+			+	+		
24h Transport			+	+			+	+			+	+
24h Recovery	+	+	+	+	+	+	+	+	+	+	+	+
48h Recovery	+	+	+	+	+	+	+	+	+	+	+	+
72h Recovery	+	+	+	+	+	+	+	+	+	+	+	+

[a] + indicates where faecal samples were collected

9.5.2 Microbiological testing of samples

Only 10g of faeces was analysed when more than 10g of faeces was collected, when less than 10g was present, all of the faecal material were processed as follows. If less than 0.5g of faeces was present, the sample was treated as a swab. Faeces were diluted 1/10 with buffered peptone water (BPW), mixed using a bag mixer and 3ml was removed and stored for enumeration of pathogens. Swabs were processed by adding 10ml of BPW to the container and vortexed for 1 min to mix. For the purpose of enumeration, swabs were considered to contain 0.1g of faeces.

9.5.2.1 Enumeration of E. coli

Faecal and swab samples were serially diluted in BPW and plated onto Petrifilm™ *E. coli*/Coliform Count Plate (3M). Plates were incubated at 37°C for 24h and enumerated following the manufacturer's instructions.

9.5.2.2 Enumeration and presence of pathogens

Presence or absence of *E. coli* O157 and *Salmonella* was determined by enriching for 6 h at 42°C followed by testing using Dynabeads anti-*E. coli* O157 and Dynabeads anti-*Salmonella* (Dynal, Oslo, Norway) with Automated Immunomagnetic Separation (AIMS) following previously described protocols (Fegan et al. 2004b; Fegan et al. 2005). Enumeration of *E. coli* O157 and *Salmonella* in samples which tested positive was performed using a Most Probable Number and AIMS protocol (Fegan et al. 2004a; Fegan et al. 2004b).

9.5.2.3 Prevalence of Shiga toxin genes

DNA templates were prepared from each faecal sample by preparing a boiled cell lysate of the enrichment used for detection of *E. coli* O157 and *Salmonella*. Briefly, 1ml of enriched faeces was centrifuged at 17,000g for 3 min. The supernatant was removed and the pellet was resuspended in 1ml of sterile distilled water (SDW). The samples were centrifuged again (3 min at 17,000g) and the supernatant was removed. The pellet was resuspended in a final volume of 500µl of SDW and held in a heating block at 98°C for 10 min. The sample was mixed gently and centrifuged for 3 min at 17,000g. The supernatant was transferred to a fresh 1.5ml tube and stored at -20°C and used as DNA template in polymerase chain reactions (PCR). The presence of Shiga toxin genes (*stx*) was determined using the PCR protocol of Paton and Paton (Paton and Paton 1998) where only the primers targeting stx_1 or stx_2 were used. Separate PCR reactions were used for detecting each type of *stx*.

9.5.3 Statistical analysis

All counts were converted to $\log_{10}$ for statistical analysis. Results were analysed using the statistical computer package Minitab (Mintab Inc, PA). Swab samples were treated as if they contained 0.1g of faeces. When *E. coli* could not be enumerated, the number was considered to be one half the limit that could be determined. A one-way ANOVA was used to determine if there were differences in *E. coli* counts after different transport, curfew and recovery times. A two-way ANOVA was used to determine if there was any interaction between the different curfew and transport times on counts of *E. coli*. The Chi-square test was used to determine if the prevalence of Shiga toxin genes in the

faeces of sheep differed between treatments. Results were considered significantly different if $p<0.05$.

9.6 Results

A total of 680 faecal samples were analysed during April and May 2007. *E. coli* was enumerated in all but 3 faecal samples. In two of these faecal samples, *E. coli* were overgrown by coliforms (coliform counts of $\log_{10}$ 5.11 and 7.46 cfu/g). At the lower dilution the plates were too overgrown to provide any counts of either *E. coli* or coliforms. For one sample there were no coliforms or *E. coli* detected on the Petrifilm plates. Of the 680 samples collected from the sheep, faeces were obtained for 619 samples and 61 swabs were taken. There were 34 outliers in relation to counts of *E. coli*, 3 of which were from swabs, therefore the swab samples were included in the statistical analysis.

9.6.1 Enumeration of *E. coli*

The counts of *E. coli* in the faeces of sheep ranged from $\log_{10}$ 1.7 to 8.61 cfu/g with an overall mean of $\log_{10}$ 6.05 cfu/g (SD 1.07cfu/g). The mean $\log_{10}$ counts of *E. coli* in the faeces of different groups of sheep after different treatments are shown in Figure 1. The mean $\log_{10}$ count of *E. coli* in the faeces of sheep from different groups prior to treatment ranged from 4.93 to 6.86 cfu/g, while after 72h recovery the counts ranged from 5.11 to 6.61 cfu/g. This variability continued throughout the treatments without any clear trends or significant relationships being observed for the effect of FWD or transport times on mean *E. coli* counts. The highest mean $\log_{10}$ count of *E. coli* was found in group 6 sheep after 24h transport ($\log_{10}$ 7.6 cfu/g) and the lowest was from group 12 after 48h recovery ($\log_{10}$ 4.47cfu/g). Both of these groups of sheep were subjected to 24h FWD and 24h transport, indicating that these FWD and transport treatments may cause fluctuations in the mean $\log_{10}$ counts of *E. coli*, but not in a consistent pattern associated with FWD or transport. The two-way ANOVA analysis on 48h recovery versus FWD and transport times indicated a small effect of transport on *E. coli* counts after 48h ($p<0.1$) and some interaction between the mean $\log_{10}$ counts of *E. coli* after 48h recovery and the period of FWD ($p=0.01$).

The changes in mean $\log_{10}$ counts of *E. coli* in the faeces of sheep from different treatments are shown in Figure 2. The variation in counts was less than a 1 log change either above or below the level prior to any treatment. It appears that in some cases the mean $\log_{10}$ *E. coli* count would increase after a treatment (e.g. groups 6/12 after 24h transport), while the same treatment for a different group would result in a decrease (e.g. groups4/10 after 24h transport). This was the case for mean $\log_{10}$ *E. coli* counts after 48h and 72h recovery and is probably why there was a small interaction observed

between 48h recovery and FWD. Mean $\log_{10}$ *E. coli* counts after 24h recovery appeared in most cases to be higher or equal to those before any treatment, however this was not significant.

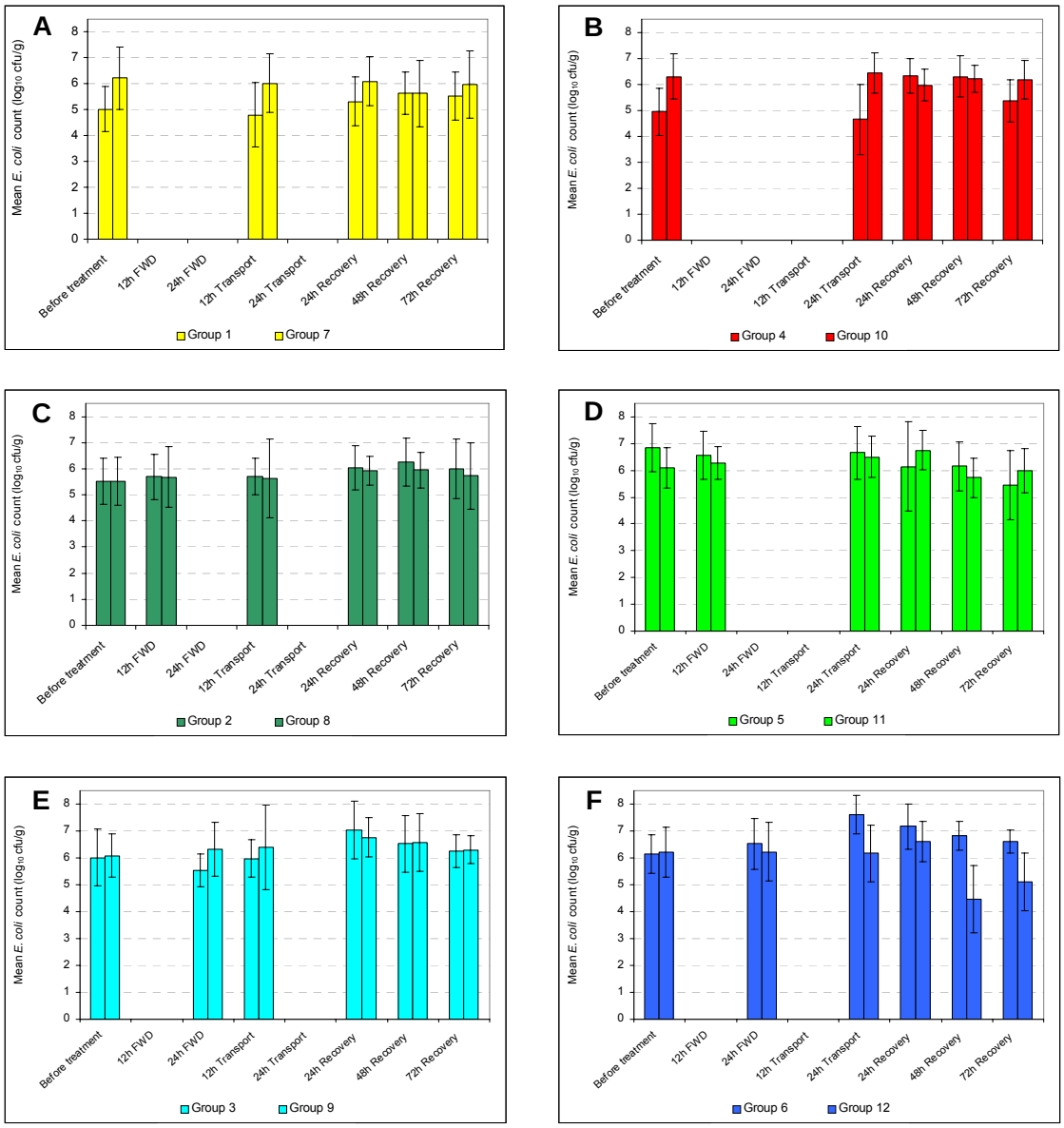


Figure 1. Mean $\log_{10}$ *E. coli* counts in the faeces of sheep in different groups after FWD and transport treatments (treatments were performed in duplicate). Mean $\log_{10}$ *E. coli* counts in the faeces of sheep which were not treated with FWD but were transported for 12h (A) and 24h (B) , 12h FWD with 12h (C) and 24h (D) transport and 24h FWD with 12h (E) and 24h (F) transport.

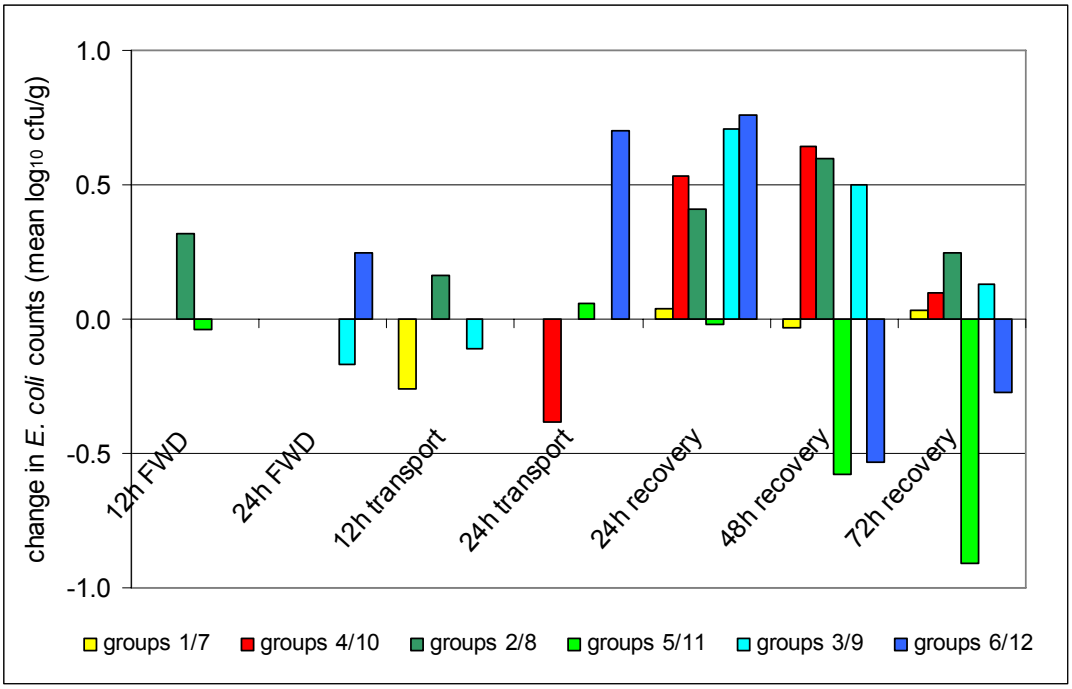


Figure 2. Change in mean $\log_{10}$ counts of *E. coli* in the faeces of sheep after different treatments.

Statistical analysis of the data revealed no other significant differences or interactions of FWD or transport times on the numbers of *E. coli* detected in the faeces of sheep. This is clearly demonstrated when all the data from different treatments are combined (Figure 3). The mean *E. coli* counts in the faeces of sheep varied from $\log_{10}$ 5.76 cfu/g (after 12h transport) to 6.35 cfu/g (after 24h recovery). This was a range of $\log_{10}$ 0.59 cfu/g across all treatments from samples collected prior to treatment, after different FWD and transport treatments and after recovery.

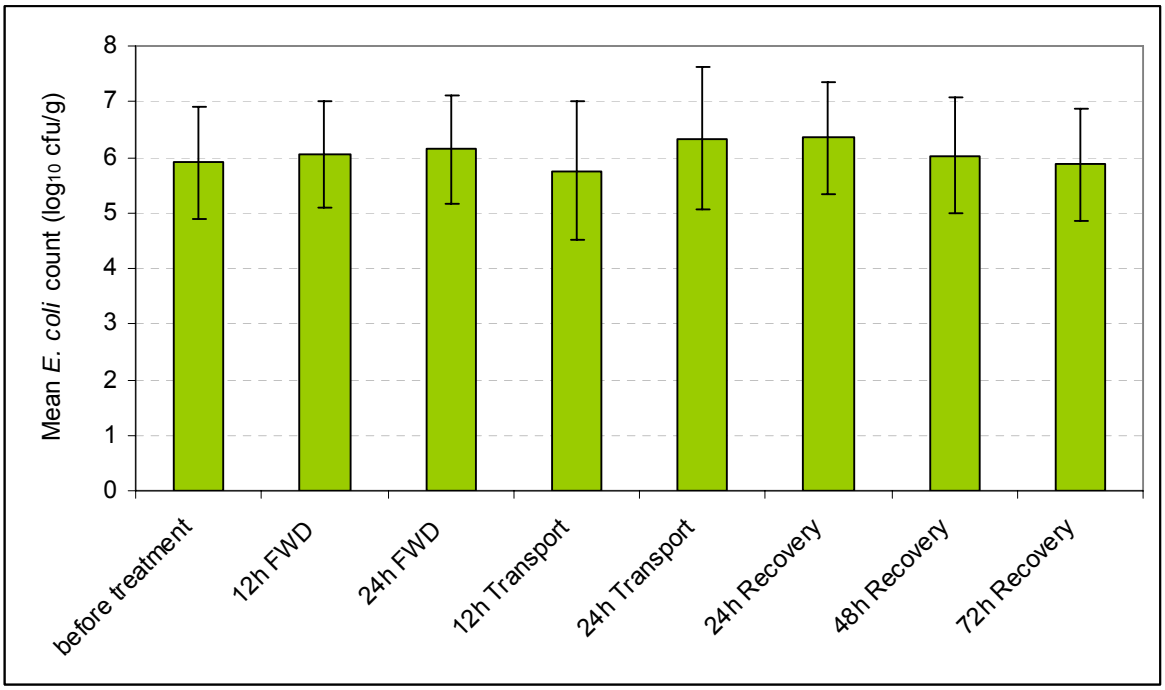


Figure 3. Mean $\log_{10}$ *E. coli* counts in the faeces of sheep when combined for each treatment.

9.6.2 *E. coli* O157 and *Salmonella*

E. coli O157 and *Salmonella* were not detected in any faecal samples with the methods used in this study.

9.6.3 Prevalence of Shiga toxin genes

Shiga toxin genes were detected in 561 (83%) of 679 sheep faeces tested (one sample was lost and not tested). Overall stx_1 was found in 534 (79%) of samples and stx_2 in 341 (50%). Both *stx* genes were detected in 322 (47%) faecal samples, stx_1 only in 216 (32%) and stx_2 only in 23 (3%). The changes in prevalence of stx_1 and stx_2 in the faeces of sheep after different treatments are shown in Figure 4 and Figure 5 respectively. There was only one significant difference found which was for animals treated with 24h FWD and 12h transport, (groups 3 and 9) which had a significantly lower prevalence of stx_1 after 12h transport than at any other time (p<0.05). No other interactions or correlations were found which may indicate this result was an anomaly. The variability of replicates was often high with prevalence ranges of 30% to 90% occurring with a replicate.

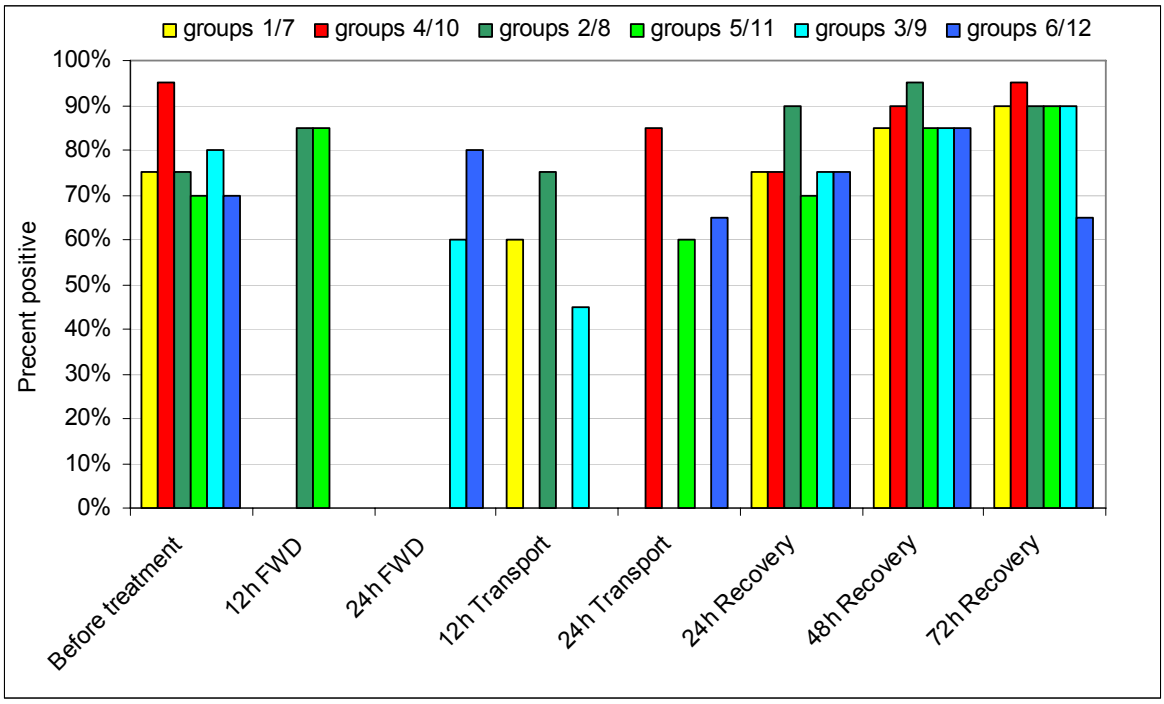


Figure 4. Prevalence of stx_1 in the faeces of sheep after different treatments. Coloured boxes represent different treatments and the numbers indicate the sheep group.

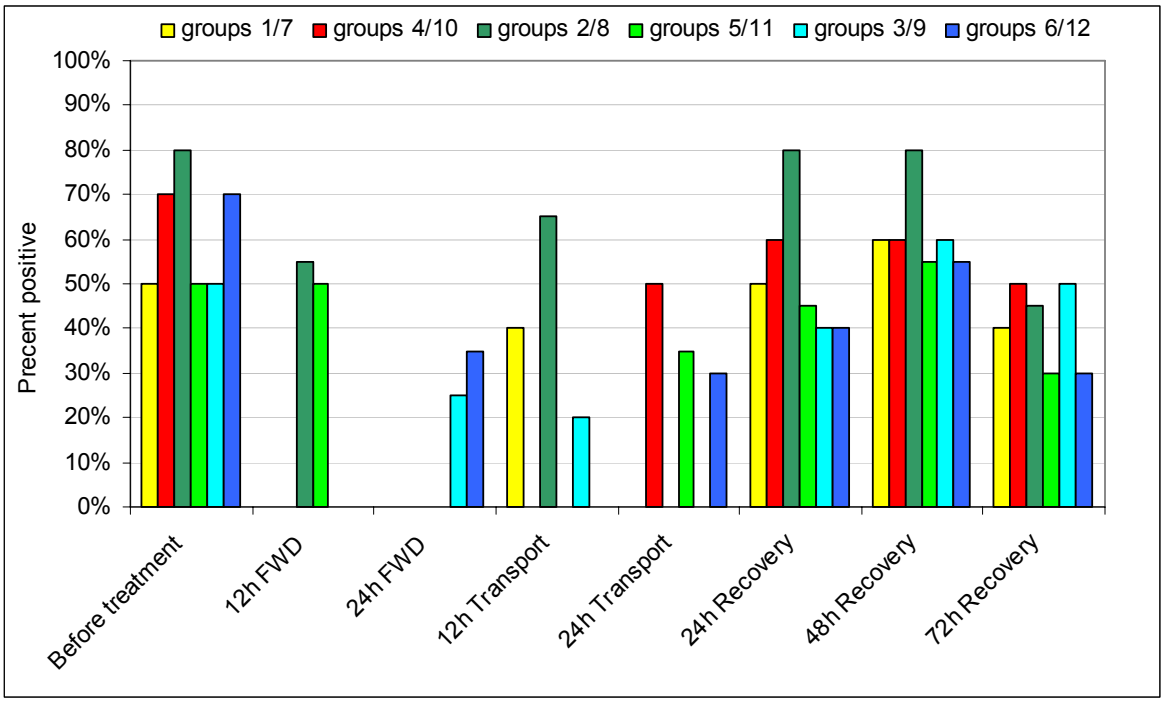


Figure 5. Prevalence of stx_2 in the faeces of sheep after different treatments. Coloured boxes represent different treatments and the numbers indicate the sheep group.

In general there was a trend for stx_1 prevalence to decrease after 12 and 24h transport and increase after 48 and 72h recovery (Figure 6), but these differences were not significant. The prevalence of stx_2 also decreased during FWD and transport treatments to increase again after 24 and 48h curfew (Figure 6), but these differences were not significant.

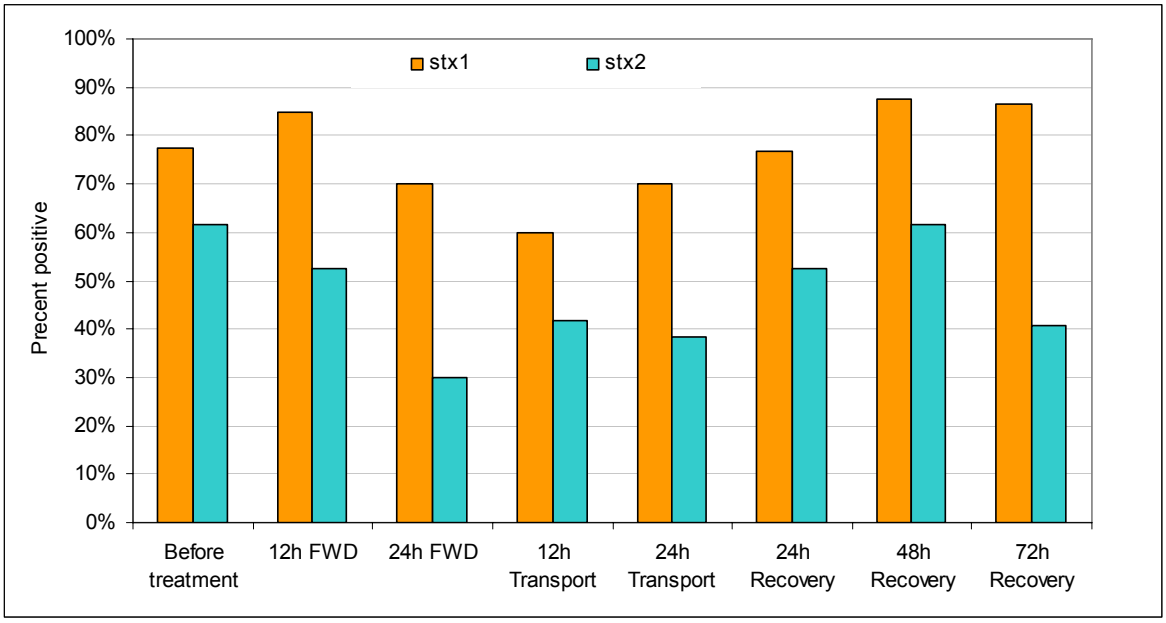


Figure 6. The prevalence of stx_1 or stx_2 or both genes in the faeces of sheep after different treatments

9.7 Discussion

E. coli counts were determined in 677 of the 680 faecal samples collected. The mean count of *E. coli* in faecal samples was $\log_{10}$ 6.05 cfu/g, with a minimum of 1.7 cfu/g and a maximum of 8.61 cfu/g. These counts are similar to those reported in a recent study where faecal *E. coli* numbers were determined for sheep at slaughter, with a mean count of $\log_{10}$ 6.23 cfu/g, minimum of 3 cfu/g and maximum of 8.81 cfu/g (A.MFS.0060). Mean $\log_{10}$ *E. coli* counts in sheep faeces fluctuated throughout the trial (range 4.47 to 7.6 cfu/g) regardless of treatment as there appeared to be no consistent effect of FWD, transport times or recovery times. Statistical analysis revealed only one significant interaction which was between counts after 48h recovery and FWD. However, this result was not considered to be relevant as it may have been skewed by the results of sheep groups 6 and 12 (e.g. highest mean count of *E. coli* in any group of sheep which occurred after 24h transport and lowest mean count which occurred after 48h recovery).

There were no pathogens detected in the faeces of animals enrolled in this study. The pathogens may have been present in levels below those that could be detected using the methods applied in this study. The prevalence of *E. coli* O157 and *Salmonella* in the faeces of different groups of sheep can be quite variable. *E. coli* O157 tends to be

isolated less frequently from sheep than cattle, with sheep isolation rates generally below 10% (Chapman et al. 1997; Chapman et al. 2001; Keen et al. 2006; Zweifel et al. 2006). The prevalence of *Salmonella* also tends to be variable with between 0.1 and 42% of sheep reported positive for this organism (Samuel et al. 1981; Davies et al. 2004; Zweifel et al. 2004; Branham et al. 2005). *E. coli* O157 and *Salmonella* were isolated from the faeces of animals in 2 of 5 groups of sheep tested in a recent study from Australia (A.FMS.0060). The numbers of *E. coli* and *Salmonella* in the faeces of sheep starved for 3 days were found to increase during starvation, then decrease again on return to normal feeding (Grau et al. 1969). The current study only used 12 and 24h starvation and it is possible that FWD for longer periods may have resulted in significant increases in the *E. coli* populations and possibly the detection of *Salmonella*. However, such long periods of FWD are unlikely to occur in current industry practice.

There was only one significant effect of FWD and transport treatments on the prevalence of *stx* in the faeces of sheep, which was a significantly lower prevalence of stx_1 in the faeces of sheep after 12h transport following a 24h FWD period. The variability between different groups of sheep may indicate this result is an anomaly. In general, the prevalence of both Shiga toxin genes tended to decrease after FWD and transport, but the results were not significant. The majority of faeces contained both stx_1 and stx_2, which is consistent with the populations of STEC obtained from sheep in Australia which tend to carry both stx_1 and stx_2 genes (Fegan and Desmarchelier 1999; Djordjevic et al. 2001).

9.8 Conclusions

There appears to be little effect of FWD or transport treatments for the times studied in these experiments on the mean $\log_{10}$ counts of *E. coli* in the faeces of sheep indicating that these practices will have little impact on the shedding of *E. coli* in the faeces of sheep. The prevalence of *stx* in the faeces of sheep was not significantly affected by the FWD or transport treatments. The impact of FWD and transport up to 24h is minimal on the numbers of *E. coli* shed and the prevalence of *stx* in the faeces of sheep.

9.9 Acknowledgements

The assistance of staff at CSIRO Livestock Industries, Armidale was vital for providing the experimental sampling plan, organising the FWD and transport treatments, ensuring collection of samples from animals for this study.

9.10 References

Branham, L.A., Carr, M.A., Scott, C.B. and Callaway, T.R. (2005) *E. coli* O157 and *Salmonella* spp. in white-tailed deer and livestock. *Curr Issues Intestinal Microbiol* **6**, 25-29.

Chapman, P.A., Cerdan Malo, A.T., Ellin, M., Ashton, R. and Harkin, M.A. (2001) *Escherichia coli* O157 in cattle and sheep at slaughter, on beef and lamb carcasses and in raw beef and lamb products in South Yorkshire, UK. *Int J Food Microbiol* **64**, 139-150.

Chapman, P.A., Siddons, C.A., Cerdan Malo, A.T. and Harkin, M.A. (1997) A 1-year study of *Escherichia coli* O157 in cattle, sheep, pigs and poultry. *Epidemiol Infect* **119**, 245-250.

Davies, R.H., Dalziel, R., Gibbens, J.C., Wilesmith, J.W., Ryan, J.M., Evans, S.J., Byrne, C., Paiba, G.A., Pascoe, S.J. and Teale, C.J. (2004) National survey for *Salmonella* in pigs, cattle and sheep at slaughter in Great Britain (1999-2000). *J Appl Microbiol* **96**, 750-760.

Djordjevic, S.P., Hornitzky, M.A., Bailey, G., Gill, P., Vanselow, B., Walker, K. and Bettelheim, K.A. (2001) Virulence properties and serotypes of Shiga toxin-producing *Escherichia coli* from healthy Australian slaughter-age sheep. *J Clin Microbiol* **39**, 2017-2021.

Fegan, N. and Desmarchelier, P. (1999) Shiga toxin-producing *Escherichia coli* in sheep and pre-slaughter lambs in eastern Australia. *Lett Appl Microbiol* **28**, 335-339.

Fegan, N., Higgs, G., Vanderlinde, P. and Desmarchelier, P. (2004a) Enumeration of *Escherichia coli* O157 in cattle faeces using most probable number technique and automated immunomagnetic separation. *Lett Appl Microbiol* **38**, 56-59.

Fegan, N., Higgs, G., Vanderlinde, P. and Desmarchelier, P. (2005) An investigation of *Escherichia coli* O157 contamination of cattle during slaughter at an abattoir. *J Food Prot* **68**, 451-457.

Fegan, N., Vanderlinde, P., Higgs, G. and Desmarchelier, P. (2004b) Quantitation and prevalence of *Salmonella* in beef cattle presenting at slaughter. *J Appl Microbiol* **97**, 892-898.

Grau, F.H., Brownlie, L.E. and Smith, M.G. (1969) Effects of food intake on numbers of *Salmonella* and *Escherichia coli* in rumen and faeces of sheep. *J Appl Bacteriol* **32**, 112-117.

Keen, J.E., Wittum, T.E., Dunn, J.R., Bono, J.L. and Durso, L.M. (2006) Shiga-toxigenic *Escherichia coli* O157 in agricultural fair livestock, United States. *Emerg Infect Dis* **12**, 780-786.

Knowles, T.G. (1998) A review of the road transport of slaughter sheep. *The Veterinary Record* **2**, 212-219.

Kudva, I.T., Hatfield, P.G. and Hovde, C.J. (1995) Effect of diet on the shedding of *Escherichia coli* O157:H7 in a sheep model. *Appl Environ Microbiol* **61**, 1363-1370.

Paton, A.W. and Paton, J.C. (1998) Detection and characterization of Shiga toxigenic *Escherichia coli* by using multiplex PCR assays for stx1, stx2, eaeA, enterohemorrhagic *E. coli* hlyA, rfbO111, and rfbO157. *J Clin Microbiol* **36**, 598-602.

Samuel, J.L., Eccles, J.A. and Francis, J. (1981) *Salmonella* in the intestinal tract and associated lymph nodes of sheep and cattle. *Journal of Hygiene* **87**, 225-232.

Zweifel, C., Kaufmann, M., Blanco, J. and Stephan, R. (2006) Significance of *Escherichia coli* O157 in sheep at slaughter in Switzerland. *Schweiz Arch Tierheilkd* **148**, 289-295.

Zweifel, C., Zychowska, M.A. and Stephan, R. (2004) Prevalence and characteristics of Shiga toxin-producing *Escherichia coli*, *Salmonella* spp. and *Campylobacter* spp. isolated from slaughtered sheep in Switzerland. *Int J Food Microbiol* **92**, 45-53.