finalreport

FEEDLOT

Project code: B.FLT.0345

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Date published: March 2009

ISBN: 9781741914665

PUBLISHED BY

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Assessment of Betaine and Glycerol as ameliorants of heat load in feedlot cattle

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

Abstract

The primary purpose of this study was to investigate the effect of betaine included at 0, 10, 20 and 40 g/head/day; and to investigate the effect of glycerol fed at an inclusion rate of 5% of dry matter intake on core body temperature and respiratory dynamics of feedlot steers over the summer months (120 days on feed). The dietary treatments were replicated across shaded and non-shaded pens. The study reports on these findings and the treatment impacts on animal productivity and animal welfare with associated economic production benefits.

The major findings from this study are: (i) provision of shade has economic ($40.66/per head) and welfare benefits over the summer months, (ii) addition of betaine did not improve performance or welfare outcomes, and (iii) addition of glycerol had a positive effect on HSCW but was not a cost effective supplement.

This study confirms that shade is the primary method for alleviation of heat load in black *Bos taurus* cattle.

Executive summary

The objectives of this project were to:

1. Identify and document the relationship between steer core body temperature, physical animal response (respiration rate/panting score/blood chemistry; meat quality) and climatic parameters during heat stress events in a feedlot field environment with or without access to shade.
2. Identify and document the relationship between steer core body temperature and measurements of animal production, during heat stress events in a feedlot field environment.
3. Report on the effect of betaine included at 0, 10, 20 and 40 g/head/day; and to investigate the effect of glycerol fed at an inclusion rate of 5% DMI to minimise the increase in steer core body temperature and its resultant impact on animal productivity and animal welfare with associated economic production benefits.
4. Further quantify and revise climatic predictors of heat stress as identified in the project data.
5. Further quantify the effect of shade on alleviation of heat stress.

The study was conducted between the 10 September 2007 and the 13 March 2008 using 164 Angus steers (396 kg non fasted liveweight at induction). The steers were on feed for 120 days, from 12 November 2007 until 11 March 2008. Five dietary treatments and two shade treatments were used in a replicated study of two pens per treatment. The treatments (T) were:

- T1 = control – 0 g/hd/d betaine - no shade
- T2 = control – 0 g/hd/d + shade
- T3 = betaine – 10 g/hd/d betaine - no shade
- T4 = betaine – 10 g/hd/d betaine + shade
- T5 = betaine – 20 g/hd/d betaine - no shade
- T6 = betaine – 20 g/hd/d + shade
- T7 = betaine – 40 g/hd/d betaine - no shade
- T8 = betaine – 40 g/hd/d + shade
- T9 = glycerol (as 5% dry matter intake) – no shade
- T10 = glycerol (as 5% dry matter intake) + shade

Results of the study
The findings from this study provide for the first time a scientific basis to the use of betaine and glycerol in diets fed to finishing cattle over the summer months in Australian feedlots. This studies suggest that there is no benefit of adding betaine or glycerol to the diets of *Bos taurus* feedlot cattle as a method of heat alleviation over the summer months.

A number of clear positive, measurable welfare outcomes (reduction in core body temperature, and reduction in mean panting score) and production responses have been demonstrated when shade is used for Angus cattle over the summer months. In the current study shaded improved returns by $40.69 per head over 120 day feeding period.

The use of shade will not only improve animal welfare, and will improve public perception of the welfare of feedlot cattle. These will have both short and longer term benefits for the feedlot industry.

The conclusions from the study are as follows:

1. Betaine inclusion in the diet did not improve performance or reduce the impact of high heat load. This finding was unexpected given the positive responses in animal house studies and the anecdotal evidence form commercial feedlots. We can only speculate that there may be dietary ingredient interactions which may reduce the efficacy of betaine.
2. Glycerol inclusion in the diet did not reduce the impact of high heat load.
3. There was a positive response of feeding glycerol in terms of HSCW, however the high cost of freight associated with glycerol in the current study (essentially doubled the cost of glycerol) resulted in a dollar return that was below the control.
4. Access to shade reduced the impact of extreme conditions but did not completely eliminate heat stress.
5. The cattle increased shade usage when the HLI>86. This suggests that the Risk Analysis Program thresholds are correct for the reference animal.
6. The relative humidity value in the HLI equation appears too high when black globe temperature is below 25ºC. This results in an overestimation of the impact of climatic conditions (AHLU) especially in the mornings.
7. During periods of high heat load cattle with access to shade had lower midday mean panting scores (20 to 30% lower). This indicates that the cattle with access to shade do not need to 'work' as hard to maintain body temperature via panting.
8. During periods of high heat load cattle with access to shade had less variation in mean body temperature (0.9 – 1.5ºC) compared to cattle without access to shade (1.5 – 2.6ºC). Maximum body temperature were greater for non-shaded *cv.* shaded cattle (41.7ºC and 40.5ºC respectively). These results suggests that shaded cattle are better able to regulate body temperature because they are not exposed to the maximum solar load.
9. There was considerable individual variation in terms of body temperature and panting responses to high heat load.
10. The shaded cattle had a better feed efficiency than did the un-shaded cattle at 6.25:1 and 6.60:1 respectively. Based on a 100 kg weight gain and feed at $300/t, the cost of feeding the non-shaded cattle was $12.25 greater than the shaded cattle.
11. Shaded cattle had lower dressing percentage but overall higher HSCW (6 kg) than non-shaded cattle.
12. Based on conclusion 10, the shaded cattle returned $28.44 per head more than the non-shaded cattle. When conclusion 9 is included the shaded cattle returned $40.69 per head more than the non-shaded cattle.
13. Cattle fed glycerol had a greater dressing percentage than the other dietary treatment groups.
14. Land transport did not adversely impact on body temperature. The cattle on the top deck had a higher body temperature than those on the lower deck. This was most likely due to the effect of solar load.

Based on the results from this study the following recommendations have been made.

Recommendation 1: Shade should be considered as the primary method to alleviate heat load for black *Bos taurus* feedlot cattle in areas were high heat load is expected. Shade will improve animal welfare and production.

Recommendation 2: The findings from the study be disseminated to the feedlot sector before summer 2009/10.

Recommendation 3: The HLI equation will need to be modified to reflect the lesser impact of relative humidity when the black globe temperature is less than 25ºC. This should be undertaken in conjunction with Recommendation 4, prior to summer 2009/10.

Recommendation 4: Further statistical analysis should be undertaken of the data in order to further understand the physiological responses of cattle to high heat load. This should include data collected from previous heat load studies. The information obtained from this would further strengthen the heat load model.

Recommendation 5: Based on the greater HSCW of the cattle fed glycerol it is recommended that a replicated study be undertaken to further investigate the effects of feeding glycerol. (This recommendation is based on the assumption that the expansion of the ethanol industry in Queensland will result in glycerol being locally available resulting in a reduction in freight costs).

Contents

1 Background

1.1 Introduction

The Australian feedlot industry continues to review heat stress reduction methodologies to maintain animal production and enhance animal welfare through minimizing heat load event related morbidities and mortalities.

Heat load has a considerable impact on the productivity and welfare of livestock. The Australian feedlot industry has adopted several strategies to reduce this impact including use of environmental stress indicators (temperature humidity index, the heat load index and the accumulated heat load units), animal stress indicators (panting score/respiration rate), reduced stocking density, provision of shade, improved pen manure management and feeding management (once a day feeding in the afternoon, use of a heat stress ration). These practises are designed to improve physical and metabolic comfort of cattle during a heat stress event. However, there remain many gaps in the understanding of how cattle react to their environment (core body temperature and physical signs of stress) and the use of feed products, such as betaine and glycerol, that may be capable of improving the coping capacity of cattle.

Glycerol (registered as feed additive by the EU - E422) is a natural liquid substance with a sweet taste. Glycerol has the potential to reduce acidosis, maintain feed intake and maintain water balance in cattle. A number of studies have been published in regards to dairy cows (e.g. Fisher *et al*. 1973; Sauer *et al*. 1973; DeFrain *et al.* 2004; Reichel *et al.* 2006), and glycerol is being fed commercially to both dairy and beef cattle in Australia. However its usefulness in alieviating heat stress in beef cattle has not been studied.

Betaine (trimethylglycine) is a natural osmolyte which has an energy sparing effect which means it could improve growth performance, and change fat distribution within the animal (Saunderson and Mackinlay 1990; Eklund *et al*. 2005). Supplementing a diet with betaine may also reduce the effects of heat stress in feedlot cattle. Betaine supplementation has been observed to reduce heat stress and enhance performance and carcass quality of livestock (Eklund *et al*. 2005; Loest *et al.* 2002), however the evidence is not strong.

Because of the interest in possible heat stress suppression properties of betaine a climatically controlled study using Angus steers in individual pens for a 5 day period of continual temperature humidity index (THI) above 90 for 8 hours per day (Gaughan *et al*. 2005). Supplementing the diet with betaine resulted in a significant reduction in the heat stress response. This was seen by a lower increases in core body temperature and respiration rates for control versusthe supplemented steers. Additional responses were observed through increased dry matter intake in the supplemented steers. Over the 2005 – 2006 summer period an unreplicated pilot study was conducted at the Brigalow Research Station (Loxton *et al*. 2007). The pilot study assessed the efficacy of *Bos Koolus*® (betaine supplement) in ameliorating the impact of environmental heat stress for 100 days from December 2005 to late March 2006 by monitoring core body temperature (implanted intrrabdominal temperature transmitters) of Angus steers fed for the short fed export markets. The study consisted of 32 steers monitored in 2 feedlot pens, fed either a control or *Bos Koolus*® ration (BK) which consited of betaine, sodium bicarbonate and potassium carbonate. Core body temperatures were recorded at 15 minute intervals throughout the study period. Mean core body temperature for both treatments from Day 30 to Day 60 was 39.09°C with significant variation between individual animals in both treatments. Preliminary data analysis trends suggest BK marginally improved DMI, water intake, ADG and hot standard carcase weight and net carcase value.

Glycerol has been fed to dairy cows for some time. However its use in beef feeding programs is new. It is used in human atheletes as a means of maintating water balance, and also as an energy source. The hypothesis is that glycerol wil help maintain water balance and thereby reduce the effects of heat stress. The application of betaine and glycerol to ameliorate heat stress in feedlot cattle and improve performance and carcass weight and quality at different dosages has not been evaluated in a field situation. The proposed study will investigate the dose response of betaine, and also investigate the effects of betaine with and without shade and glycerol with or without shade. It has been suggested that shade be manadatory for all feedlots in Australia.

The Risk Assessment Program (RAP) which has been developed for the Australian feedlot industry shows that shade may not be required where *Bos indicus* animals are used, and that nutritional management may offset the need for shade in some areas where *Bos taurus* cattle are used. The RAP model suggests that heat load can be managed by the use of shade and/or nutrition. The efficacy of betaine or glycerol in ameliorating the effects of high heat load need to be studied in cattle with and without access to shade. The first phase of a shade study was completed at UQ Gatton over summer 2006/07 under similar environmental conditions expected at Brigalow during summer.

1.1.1 Previous research

There has been a number of research projects conducted in the area of heat load management in the Australian feedlot industry. A list of previous research projects funded by Meat and Livestock Australia Ltd. is shown below.

- FLOT.307, 308 & 309 – Recommendations for reducing the impact of elements of the physical environment on heat load in feedlot cattle.
- FLOT.310 – Measuring microclimate variations in two Australian feedlots.
- FLOT.312 – Heat stress software development.
- FLOT.313 – Forecasting feedlot thermal comfort.
- FLOT.315 – Applied scientific evaluation of feedlot shade design.
- FLOT.316 – Development of an excessive heat load index for use in the Australian feedlot industry.
- FLOT.317 – Measuring the microclimate of eastern Australian feedlots.
- FLOT.327 – Reducing the risk of heat load for the Australian feedlot industry.
- FLOT.330 – Validation of the new Heat Load Index for use in the feedlot industry
- FLOT.335 – Improved measurement of heat load in the feedlot industry.
- B.FLT.0337 – Assessment of varying allocations of shade area for feedlot cattle – Part 1 120 days on feed
- B.FLT.0343 – Assessment of varying allocations of shade area for feedlot cattle – Part 2 182 days on feed

Major outputs from these projects include the development of new measures of heat load including the Heat Load Index (HLI), the Accumulated Heat Load Units (AHLU) and a computer based risk assessment package, the Risk Analysis Program (RAP).

2 Project objectives

2.1 Project objectives

The objectives of Project B.FLT.0345 were to;

1. Identify and document the relationship between steer core body temperature, physical animal response (respiration rate/panting score/blood chemistry; meat quality) and climatic parameters during heat stress events in a feedlot field environment with or without access to shade.
2. Identify and document the relationship between steer core body temperature and measurements of animal production, during heat stress events in a feedlot field environment.
3. Report on the effect of betaine included at 0, 10, 20 and 40 g/head/day; and to investigate the effect of glycerol fed at an inclusion rate of 5% DMI to minimise the increase in steer core body temperature and its resultant impact on animal productivity and animal welfare with associated economic production benefits.
4. Further quantify and revise climatic predictors of heat stress as identified in the project data.
5. Further quantify the effect of shade on alleviation of heat stress.

3 Methodology

3.1 Animal ethics approval

This project was approved (SA 2007/06/2002) by the Queensland Department of Primary Industries and Fisheries, Staff Animal Experimentation Ethics Committee.

3.2 Methodology

3.2.1 Study design and treatments

A feedlot study was conducted between the 10 September 2007 and the 13 March 2008 using 164 Angus steers (396 kg non fasted liveweight at induction). The steers were on feed for 120 days, from 12 November 2007 until 11 March 2008 and were targeted at the Short-fed export beef market.

Five dietary treatments and two shade treatments were used in a replicated study of two pens per treatment. The treatments (T) were:

- T1 = Control – 0 g/hd/d betaine - no shade
- T2 = Control – 0 g/hd/d + shade
- T3 = Betaine 10 – 10 g/hd/d betaine - no shade
- T4 = Betaine 10 – 10 g/hd/d betaine + shade
- T5 = Betaine 20 – 20 g/hd/d betaine - no shade
- T6 = Betaine 20 – 20 g/hd/d + shade
- T7 = Betaine 40 – 40 g/hd/d betaine - no shade
- T8 = Betaine 40 – 40 g/hd/d + shade
- T9 = Glycerol (as 5% dry matter intake) – no shade
- T10 = Glycerol (as 5% dry matter intake) + shade

3.2.2 Study period time sequence terminology

The study period commenced on 12 November 2007 when steers were inducted into their treatment pens and fed their first ration in the PM of that day. That day is referred to as day 0 or Days on Feed (DOF) 0. The study concluded on 11 March 2008 at day 120. Wherever a day number is used in the report, it will be relative to the study commencement on November 12.

3.2.3 Animals and feedlot description

3.2.3.1 Animals

One hundred and seventy-seven Black Angus steers aged 12 – 15 months of age and of mean non fasted liveweight 378 kg were purchased from a single source in Goulburn, NSW. The steers were purchased from a single source to reduce genetic variability. The steers were transported from Goulburn to the Brigalow Research Station (BRS), via Theodore, Queensland via road, arriving 5 September 2007. Wet conditions meant that the cattle were off loaded at Moura Saleyards (46 km from BRS) were they remained for 4 days before being transported to BRS, with all steers being received at BRS by 9 September 2007.

The vaccination protocol followed post arrival for each steer was:

6 September 2007 – 2 ml trivalent tick fever vaccine (mixed bovine blood containing *Babesia bovis* & *Anaplasma centrale & Babesia bigemina).* DPI Tick fever Centre, Wacol Queensland. Vaccinated while at Moura Saleyards.

11 September 2007 – 2 ml 1° dose Webster's Bovine Ephemeral Fever Vaccine (Living) (® Registered Trademark). Fort Dodge Australia Pty Ltd.
- 2.5 ml Longrange® Botulinum Vaccine (Toxoids from prepared cultures of both *Clostridium. Botulinum* type C and *Cl. Botulinum* type D). Pfizer Australia Pty Ltd.
- 1ml/100kg liveweight Cydectin® Pour On for cattle and red deer (5g/l moxidectin solvent, 150 g/l hydrocarbon liquid). Fort Dodge Australia Pty Ltd

24 September 2007 - 2 ml 2° dose Webster's Bovine Ephemeral Fever Vaccine (Living) (® Registered Trademark). Fort Dodge Australia Pty Ltd.

Of the 177 steers purchased 13 were not used in the study. These animals were removed due to poor temperament, health problems or because their live weight was outside of the desired range. Twelve of the 'cull' animals were kept at the feedlot and fed the control ration throughout and one (poor temperament) was kept in a nearby paddock. Three of the cull steers were implanted with a body temperature transmitter (see below). This was done to ensure there were "back up" animals in case a transmitter in one of the steers within the trial failed.

The steers were not implanted with hormonal growth promotants.

3.2.3.2 Feedlot description

The BRS feedlot has 22 pens consisting of 6 pens of 168 m^2 (pens 1 – 6) and 16 pens of 144 m^2 (pens 7 – 22) (see Appendix 1), and has a north south alignment. The surface of the pens was soil. Concrete feed bunks were located at the front of each pen. The linear feed bunk area/steer was 583 mm for pens 7 – 22, and 588 mm for pens 3 – 6. Linear water trough areas were 279 mm/head for pens 7 – 22 and 242 mm/ head for pens 3 – 6. Stocking densities of 18 m^2 were obtained for pens 7 – 22 using 8 steers/pen. The stocking density of pens 3 – 6 was 19 m^2, and this was achieved using 9 steers/pen. All pens initially had shade, however for this study the shade was removed from 10 pens. These were: 5, 6, 11, 12, 13, 14, 19, 20, 21 and 22. In the shaded pens, shade was provided by shade cloth (80% solar block) located and providing a shade footprint at midday of 3.2 m^2/animal in pens 7, 8, 9, 10, 15, 16, 17 and 18. Slightly more shade (3.26 m^2 /steer) was provided in pens 3 and 4. The pen dimensions and the location of shade and water troughs are presented in Appendix 1 and Appendix 2 (specifically pens 3 – 6).

Within each dietary treatment group two pens were un-shaded, and two pens had shade (approximately 3.2 m^2/steer for control and betaine feed steers and 3.26 m^2/steer for glycerol fed cattle). The study was replicated so that there were two pens per treatment. Eight steers per pen were used for the betaine treatments (0, 10, 20 and 40 g of betaine/steer/day), and 9 steers per pen were used for the glycerol treament (as 5% dry matter intake). The differences in the number of steers per pen was necessary to ensure that stocking densities were similar (18 m^2/steer) between the betaine treatments and glycerol treatment groups (19 m^2/steer). The pens used for the glycerol treatment were larger (168 m^2) than those used for the betaine treatments (144 m^2) hence the differences in the number of animals used. The betaine control group (0 g/steer/day) also served as the control for the glycerol group.

3.2.4 Live animal phase measurement schedule of events

A description of the date and measurement schedule for the study during the live animal phase is shown in Appendix 3.

3.2.5 Allocation of cattle to surgery and to treatment pens

On 7 October, 2007 the rectal temperature, non fasted liveweight, visual hip height and temperament scores were obtained for the 177 steers. These parameters were used to select 164 trial steers and the 13 spares. The 13 steers considered as spare steers because they were considered outliers in respect to liveweight, rectal temperature, hip height and temperament. The selection of steers on rectal temperature ensured the steers selected for surgery represented the overall population of steers in respect to the range in rectal temperature. The 164 steers were then ranked and randomly allocated on rectal temperature and non fasted liveweight into two groups - one of 63 steers for surgical implantation of temperature transmitters (surgery steers) and a second group of 101 steers that would not be implanted (non surgery steers). Three of the steers intended for surgery were identified as 'spare' transmitter steers.

Following surgery and the post operative period, the 164 steers (63 steers that had been surgically implanted with temperature transmitters and the 101 steers not implanted) were measured on 11 November, 2007 for non fasted liveweight, visual hip height and visually assessed US body condition score (USBCS). Steers were grouped according to their surgery status and rectal temperature grouping (low, medium and high) and randomly allocated to pens using Genstat. The 3 'spare' surgery steers were assigned to the original 'spare' group of steers and 3 original 'spare' steers reassigned to the population of steers not surgically implanted.

There were 3 surgery steers plus 5 non surgery steers allocated per treatment pen for pens 7 – 22 and 3 surgery steers plus 6 non surgery steers allocated per treatment pen for pens 3 – 6.

The allocation of treatments to pens was constrained by the availability of shade structures. Pens 3 – 6 were used for the glycerol treatment, with 2 pens shaded (pens 3 and 4) and 2 not shaded (pens 5 and 6). In the remaining pens, shade was available in pens 7 – 10 and 15 – 18. The betaine treatments and the control were assigned at random within each set of 4 pens.

The effect of glycerol is confounded with location, and the non-glycerol part of the trial is technically a split unit design, with diet nested within shade treatment. The only way of incorporating the effects of glycerol into an analysis is to assume that the alloaction of treatment to pen was done 'at random', and that the physical location of any pen had not effect on the experimental outcome.

3.2.6 Description of body temperature transmitters and surgery

Within each pen 3 steers were implanted with an intraabdominal digital temperature transmitter (Sirtrack Ltd, Havelock North, New Zealand). Each transmitter operated on a different radio frequency (see Section 3.2.11). The radio transmissions were detected and stored on a radio reciever (TR-5 Receiver, Telonics, Mesa, Arizona, USA) until downloaded to a PC. The inclusion of 3 steers per pen with temperature transmitters resulted in a total of 63 (60 in treatments + 3 spare) steers with

transmitters for the two treatments in the study. It was considered that 3 steers with transmitters per pen will account for the variation in core body temperature between animals (as identified in the *Bos Koolus*® Pilot Study) in the statistical analysis and allow for the loss of any temperature transmitter due to malfunction as also occurred in the *Bos Koolus*® Pilot Study.

The surgical implantation of the intraabdominal digital temperature transmitters was carried out on 9 and 10, October, 2007. A full description of the surgical implantation procedure is given in Appendix 4.

The steers were monitored and inspected daily during the post operative recovery period. A veterinary inspection was carried out on 15 October, 2007 and sutures removed on 25 October, 2007. Treatment of any animals was carried out as required (Refer to Section 4.2). During the post operative recovery period in the paddock, a test period of daily temperature data acquisition was carried out to test both the implanted temperature transmitters and the receiver.

3.2.7 Induction of animals to treatment pens

The steers were walked to the yards on 11 November, 2007, for measurements of non fasted liveweight, visually assessed USBCS and hip height, collection of blood samples via the coccygeal vein of the tail and a check of the operation of the temperature transmitters.

The steers were allocated to treatment pens as per the procedure of Section 3.2.5 and held in the cattle yards overnight with access to hay and water.

During 12 November, 2007, the steers were drafted into their treatment groups and walked to their relevant feedlot pens. The steers were fed their first treatment ration in the afternoon of that day.

Refer to Appendix 3 for further details of procedures at induction.

3.2.8 Diets and feeding

The diets (including mineral supplement composition) were formulated by Integrated Animal Production (Toowoomba Qld). Mineral supplement composition and their expected analysis can be found in Appendix 5. The Betaine and Control treatments were fed the same basal diet (Table 1).

Table 1 Betaine treatment diets (expressed in kg on a 1000 kg basis)

	Starter	Intermediate 1	Intermediate 2	Finisher
Ingredients				
Wheat - dry rolled	450.0	540.0	625.0	700.0
Molasses - cane	125.0	100.0	60.0	30.0
Cottonseed meal - solvent	55.0	55.0	25.0	-
Cottonseed High Lint	70.0	80.0	80.0	90.0
Wheat straw	85.0	85.0	50.0	25.0
Sorghum silage	70.0	110.0	110.0	90.0
Lucerne hay	120.0	-	-	
Vegetable oil	-	-	10.0	20.0
Control/Betaine feedlot mineral supplement	25.0	30.0	40.0	45.0

The composition of the Control/Betaine feedlot mineral supplement is shown in Table 4.

The Control and Betaine treatments were given the relevant 5 kg Active Pack of Betaine supplement per treatment ration mixture as per the composition shown in Table 2. The 5 kg Active Pack was in addition to all other components in the ration mixt, thus the percentage of total ration mixed would be 100.5% on each occasion.

Table 2 Betaine supplement composition (expressed in kg on a 1000 kg basis)

	Control	Betaine 10g	Betaine 20g	Betaine 40g
Ingredient				
Cereal carrier	1000.0	932.7	865.3	730.0
Betaine 96%	0	67.3	134.7	270.0

The supplement manufacturing process and sampling protocol is outlined in Appendix 6.

The Glycerol treatment was fed the diet shown in Table 3.

Table 3 Glycerol treatment diet (expressed in kg on a 1000 kg basis)

	Starter	Intermediate 1	Intermediate 2	Finisher
Ingredients				
Wheat - dry rolled	423.0	509.0	598.0	676.5
Molasses - cane	95.0	75.0	30.0	-
Cottonseed meal - solvent	60.0	58.0	28.0	-
Cottonseed High Lint	70.0	80.0	80.0	90.0
Wheat straw	85.0	87.0	52.0	25.0
Sorghum silage	70.0	110.0	110.0	90.0
Lucerne hay	120.0	-	-	-
Vegetable oil	-	-	10.0	20.5
Glycerol	52.0	51.0	52.0	53.0
Feedlot mineral supplement	25.0	30.0	40.0	45.0

The composition of the Control/Betaine treatment feedlot mineral supplement and Glycerol treatment feedlot mineral supplement is shown in Table 4.

Table 4 Control/Betaine and Glycerol treatment feedlot mineral supplement composition (expressed in kg on a 1000 kg basis)

	Control/Betaine treatment feedlot mineral supplement	Glycerol treatment feedlot mineral supplement
Ingredients		
Cereal Carrier	420.9[A]	222.9
Soybean Meal	-	244.0
Limestone	362.2	360.0
Urea	86.7	120.0
Ammonium Sulphate	68.9	-
Magnesium Oxide	10.0	15.1
Salt	35.1	26.2
Potassium Chloride	4.44	-
ENC Beef-B	7.55	7.55
Rumensin 100	4.22	4.22

A Feedlot Emergency Ration (FER) which is associated with feedlot management in an extreme heat load event was fed from 7 January to 10 January 2008. The composition of that diet is shown in Table 5.

Table 5 Feedlot Emergency Ration (FER) composition (expressed in kg on a 1000 kg basis)

	Betaine FER	Glycerol FER
Ingredient		
Wheat - dry Rolled	580.0	524.0
Molasses cane	100.0	60.0
Cottonseed High Lint	90.0	90.0
Wheat straw	25.0	25.0
Sorghum silage	160.0	210.0
Glycerol		48.0
Control/Betaine treatment feedlot mineral supplement	25.0	
Glycerol treatment feedlot mineral supplement		24.0

3.2.9 Feeding management program

The feeding management used in the study was a modified 'Clean Bunk at Midday' program (Lawrence 1998). The procedures followed are outlined in Appendix 7.

The first ration was fed out in the afternoon (PM) of 12 November 2007 (Days on Feed (DOF) 0). The starter ration was used for 4 days (12/11/07 – 15/11/07), followed by intermediate 1 for 7 days (16/11/07 – 22/11/07), intermediate 2 for 6 days (23/11/07 – 28/11/07) and finisher for the remainder of the study, except for the period 07/01/08 – 10/01/08 when the heat load emergency ration (FER) was used.

3.2.10 Feed analysis

Diet grab samples and refusals were air-dried, ground to 1 mm and dry matter (DM) of both was determined by drying a sample at 100 ºC for 24 hours. These dry samples were then ashed at 500 ºC for 3 hours to determine organic matter. A sub-sample of the ground forage and refusals were freeze dried for 24 hours and these sub-samples subsequently used for nitrogen determination and spectral analysis. Nitrogen was determined using the Dumas method (vario Macro CHN/CHNS, Elementar Analysensysteme GmbH). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were estimated on an ash free basis. Metabolisable energy (ME) was estimated using the algorithm of Anon (1975).

3.2.11 Daily acquisition of body temperature data

Temperature pulses were acquired daily from a total of 62 transmitters, during livestock transport to the abattoir at Oakey and during abattoir lairage. One steer due to a post-surgery trauma related injury remained at pasture and was not included in the study.

The digital intraabdominal temperature transmitters operated in a frequency range of 150.100 to 151.360 MHz. The sampling interval of the digital temperature transmitters was 10 seconds and the resolution was 0.0625 °C i.e. if the temperature at the time of reading varied by more than the resolution from the previous reading, the transmitter sent the new temperature value to the receiver.

The initial accuracy of the transmitters is reported as ± 0.5 °C. The actual accuracy of each transmitter was determined by conducting a calibration check as per manufacturers recommended protocol prior to their implantation on 3 October, 2007 and again following recovery of the transmitters at the abattoir on 5 April, 2008, and for some transmitters on 17 April, 2008 and again on 28 April, 2008. During the study period, 2 transmitters stopped transmitting and 1 transmitter was not recovered as the steer remained at pasture. To calculate the offset, each transmitter was placed in a water bath approximating a temperature (T2) of 40.0 °C (as per laboratory mercury thermometer) and their pulse period measured. The actual temperature (T1) of each transmitter was calculated using the formula:

$$T1 = 50 – (\text{Pulse period} – 750)/50$$

An offset temperature value for each transmitter was determined as the difference between T1 and T2. All transmitters underestimated the water bath temperature (T2) with the preferred maximum offset being ± 0.5 °C based on the stated accuracy of the transmitters. The actual offsets are shown in Appendix 8. The actual range in offsets recorded was 0.58 to 0.98 °C.

From induction until January 10, 2008 (Day 59) a RFI 2.5 db gain end fed mobile dipole aerial mounted at 3 metres above ground level was used to receive radio pulses in the receiver from the implanted intraabdominal digital temperature transmitters. During this period, digital temperature pulses were often missed from implanted steers in the farthest 4 treatment pens of the feedlot because of their distance from the receiver aerial and the animal's orientation to the aerial. To overcome this problem, a RFI 4.5 db gain base station Omni vertical collinear aerial was installed on January 10, 2008 at 10 m above ground level to receive the digital temperature pulses and replace the original RFI 2.5 db gain end fed mobile dipole aerial. This aerial enhancement significantly increased the number of digital temperature pulse acquisitions in the farthest feedlot pens for the remainder of the study.

The receiver was programmed to acquire temperature pulses from every temperature transmitter steer every 30 minutes on a 24 hour cycle. At the end of each 24 hour period, temperature pulse acquisitions were stopped and data downloaded to a PC using the receiver software (TR-5 Interface software Telonics, Mesa Arizona, USA). Following download, the temperature pulse acquisitions were re-commenced for the next 24 hour period.

The downloaded data was input into a dedicated database program written specifically for the summation, daily monitoring and interpretation of temperature data for each steer. Temperatures reported were 'offset' corrected temperatures. Relevant climatic data - HLI, HLI Balance and AHLU were also imported into the 'Heat Load Database' for daily monitoring of steer welfare and for the projection of potential high heat load events.

3.2.12 Panting score data collection

Panting scores were visually assessed using the 0 – 4.5 scale, with panting score 0 being an animal under no heat load, and 4.5 being a severely heat stressed animal. The indicators for each panting score are shown in Table 6.

Table 6 Panting Score system used during data collection

Panting Score	Breathing Condition
0	No panting – normal. Difficult to see chest movement
1.0	Slight panting, mouth closed, no drool or foam. Easy to see chest movement
2.0	Fast panting, drool or foam present. No open mouth panting
2.5	As for 2 but with occasional open mouth panting, tongue not extended
3.0	Open mouth + drooling. Tongue not extended. Neck extended and head usually up
3.5	As for 3 but with tongue out slightly & occasionally fully extended for short periods + excessive drooling
4.0	Open mouth with tongue fully extended for prolonged periods + excessive drooling. Neck and head up.
4.5	As for 4.0 but head down. Cattle “breath” from flank. Drooling may cease.

(Modified from Mader *et al.* 2006).

Panting score was the key physiological and behavioural factor used in development of the HLI, and in establishing the heat load thresholds. Mean panting score was calculated according to the following formula;

$$\text{Panting Score} = \frac{\sum_{i=0}^{4.5} N_i \times i}{\sum_{i=0}^{4.5} N_i}$$

where

N_i = the number of cattle observed at panting score i

The effect of mean panting score (MPS) on cattle was assessed as follows: 0 to 0.4 minimal heat load – no stress; 0.4 to 0.8 moderate heat load – slight stress; 0.8 to 1.2 high heat load – moderate heat load; >1.2 extreme heat load cattle highly stressed (Gaughan *et al.* 2008c).

3.2.13 Exit procedures

Feedlot exit (Day 120) was on 11 March 2008. The steers were fed as normal in the afternoon of the day prior to exit (Day 119). In summary, steers were measured, drafted and loaded onto livestock transports for the journey to Oakey Abattoir, Oakey. A set procedure was followed on the day of exit which is included in Appendix 3.

3.2.14 Transport to abattoir

On 11 March 2008, at feedlot exit, following measurements, drafting, installation of temperature humidity loggers (Hobo, Onset Computer Corporation, USA) and the installation of the receiver and aerial to one of the livestock transports, the steers were loaded onto livestock transports as per the details shown in Appendices 3 and 9. In summary, the livestock transports left BRS at 1048 h and arrived at Oakey Abattoir, Oakey the same day at 1700 h.

3.2.15 Lairage and abattoir data collection procedures

Upon arrival at 1700 h at Oakey Abattoir on 11 March 2008, the steers were unloaded into lairage pens as described in Appendix 9.

The steers were kept in lairage overnight and during this time, body temperatures were being acquired from the temperature transmitter steers until approximately 0900 h on the following morning (12 March, 2008). Details of the lairage period procedures are given in Appendix 10.

The steers (n=176; 1 was retained at BRS for later sale) were slaughtered during the morning of 12 March 2008. The order of slaughter was 62 temperature transmitter steers, 104 non transmitter steers and 10 spare steers. The slaughter order within those groups was random.

Due to an auger breakdown on the plant, stunning ceased at 1104 h for 20 minutes and recommencing at 1124 h. As a consequence at the time of breakdown, bodies 1 – 39 were processed and in the carcase chiller, bodies 40 – 139 remained at various stages of dressing on the slaughter floor chain and bodies 140 – 166 were still live animals waiting stunning.

In summary, on that day tissue samples were collected, full AUS·MEAT standard carcase data collected and the carcase pH-temperature profile monitored. The digital intraabdominal temperature transmitters were recovered from the implanted steers. All carcases post dressing were held in two carcase chillers. Electrical stimulation of bodies to accelerate pH decline was not used. Full details of all slaughter procedures and data collection on this day are given in Appendix 10.

Carcases were held overnight in the Carcase Chillers according to the chiller plan shown in Appendix 10. On the morning of 13 March, 2008, a full MSA Grade Assessment was carried out, HunterLab Miniscan surface meat colour measured (Warner *et al.* 2007) and the amount of exudate (Kaufman *et al*. 1986) determined at the quartering point of the recently quartered carcase sides. Details of all chiller measurements are given in Appendix 10.

3.2.16 Meat sample collection

Striploin meat samples were collected from each left hand carcase side following chilling and chiller assessment on the morning of 13 March, 2008. These samples were collected, packed and frozen at the abattoir prior to transfer to Food Science Australia (FSA) Laboratory, Brisbane for the objective assessment of meat quality of *Longissimus dorsi* (LD) steaks dissected from the striploin samples. Samples were transported to the Food Science Australia Laboratory, Brisbane on 25 March, 2008. The striploin sample collection, storage and transport procedures are given in Appendix 10.

3.2.17 Laboratory assay of meat samples

The meat samples were received at the FSA on 25 March, 2008 in frozen state and placed in a -25°C freezer until required for assay. The procedure followed for the assay of the LD meat samples is outlined in Appendix 11. The majority of assays were as per Perry *et al*. (2001). Striploin subsamples were excised and re-frozen from the striploin for a subsequent fatty acid profile assay of adipose tissue. The procedure followed for the sub-sampling is outlined in Appendix 11. The procedure followed for the assay of the fatty acid profile of the adipose tissue from the striploin meat samples is given in Appendix 12.

3.2.18 Laboratory assay of blood parameters

Blood samples were taken from all steers with temperature transmitters (n=62) at the start and near the completion of the study (days 1 and 110). Blood samples were collected from 30 of the 62 transmitter steers on days 30, 60 and 90 when liveweight, hip height and USBCS were recorded. Blood was collected from the tail (coccygeal vein) into two 10 ml vacuum filled tubes (BD Vacutainer®, Franklin Lakes, USA) for each steer. The two tubes for each animal contained different blood anti-coagulant for separate assays. For biochemistry and hsp70 assay, tubes containing lithium heparin (anti-coagulant) were used. Immediately following collection, the whole blood samples were chilled (approximately 6 – 8˚C) before centrifugation, with plasma separated from cells within 30 min of collection. Plasma separation protocols (lithium heparin tubes) were centrifuged at 3000 rpm for 10 min. A small number of samples required a second centrifugation when plasma contained red pigmentation. Plasma was kept on ice and frozen (-20˚C) within 8 hours, and stored at -80˚C until assayed. Blood biochemistry parameters including total protein, albumin, urea, creatinine, glucose, lactate dehydrogenise (LDH), creatine phosphokinase (CPK) and chloride (Cl) were assayed using an Olympus AU400 auto analyser, adhering to the manufacturer's protocols (Olympus Australia, Mt. Waverly Victoria, Australia). Plasma globulin content was calculated as the difference between measured total protein and albumin. Sodium (Na) and potassium (K) were assayed using a Varian Spectraa 220FS Atomic Absorption Spectrometer, as per the manufacturer's specifications (Varian, Mulgrave, Victoria, Australia). For haematology analysis (whole blood), tubes containing EDTA as an anti-coagulant were used. Following collection, samples were stored at 4˚C and assayed the following day within 36 hours from when the first sample was drawn. A Cell-Dyn 3700 (Abbott Diagnostics, North Ryde, New South Wales, Australia) was used for all haematology analysis. Haematology parameters include total white cells (WBC), neutrophils, lymphocytes, monocytes, basophils, eosinophils, red cells (RBC), haemoglobin (HGB), haematocrit (HCT) and platelets.

3.2.19 HSP assay

- All diluents and buffers were brought to room temperature before use. All incubation steps were carried out in a humid container.
- Samples were diluted 1/40 in carbonate/bicarbonate buffer (TropBio, Townsville, Queensland, Australia).
- 100μl of diluted sample was added to each well of a 96 well, flat bottom microtitre plate.
- Plate was incubated overnight at room temperature (RT).
- After incubation, excess reagent was flicked out; plate was tapped dry with no wash step.
- 120 μL of post coating buffer (TropBio, Townsville, Queensland, Australia) was added to each well and incubated at RT for 2 hour.
- Excess blocking buffer was flicked out and plate was dried for 2 hour at 37°C.
- Mouse anti-human hsp70 MAb (BioScientific, Gymea, New South Wales, Australia) was diluted in TEN-TC buffer (TropBio, Townsville, Queensland, Australia) to a dilution of 1μg/ml respectively. 100 μL was added for each well of diluted MAb and incubate for 1 hour at RT.
- Excess MAb was flicked out and wells were rinsed three times with TEN-TW buffer (TropBio, Townsville, Queensland, Australia).
- Goat anti-mouse HRPO conjugated antibody (TropBio, Townsville, Queensland, Australia) was diluted in TEN-TC buffer to 1/1000. 100 μL of diluted MAb conjugate was added to each well and incubated for 1 h at RT.

- Excess MAb solution was flicked out and wells rinsed three times with TEN-TW buffer.
- 100 μL of ABTS was added to each well and incubated in the dark at RT for 1 hour.
- Optical density (OD) was measured at a dual absorbance of 414 nm and 492 nm.

NOTE: Blank control = carbonate/bicarbonate buffer.
Negative control = Foetal bovine serum (FBS) at 1/40 dilution
Positive control/protein standard at 0.04μg/ml = Human hsp70 protein (BioScientific, Gymea, New South Wales, Australia).

3.2.20 Fatty acid analysis

Adipose fat was used for analysis of fatty acid composition, derived from a sub-sample of *M. Longissimus dorsi* from which other meat quality parameters were assessed. A representative fat portion (approx. 20 – 30 mm) from the adipose fat depot was cut from over the middle of the meat sub-sample, including all adipose fat external to the meat (within the 20 – 30 mm section). The cross-section of fat within the adipose tissue was thus represented within the fat portion for assay. The fat sample was thoroughly homogenised using a knife and spatula. The methanol-choloroform step for extraction of fatty acids from meat samples was not required within this analysis since the fat sample was obtained only from the adipose tissue. Trans-esterification of the fatty acids was conducted according to the following protocol:

(i) An internal standard solution was made by weighing out 100 mg of heptadecanoic acid (C:17, margaric acid) into a 10 ml volumetric flask, dissolved in AR iso propanol and diluted to volume.

(ii) Approximately 15 – 20 mg of fat sample was weighed into a clean 25 ml volumetric flask. A positive displacement pipette was used to add 100 μL of the internal standard solution.

(iii) Methanolic NaOH was added (0.5 ml of 0.5 M), the flask was flushed with N_2 and the stopper loosely fitted in the top.

(iv) The fat samples were saponified by placing on a steam bath at 95°C for 3 – 5 min until all the sample was dissolved, avoiding taking the solvent to dryness.

(v) The samples were cooled and 2.5 ml of BF3 – methanol solution (14%) added, the flask flushed with N_2 and the stopper reinserted loosely in the top. All flasks were placed in the water bath for 1 min with the stoppers inserted firmly.

(vi) The fatty acids were esterified by heating in the steam bath for 5 min, and then cooled to room temperature.

(vii) 2 ml of heptanes was added with a Gilson positive displacement pipette and mixed.

(viii) The saturated NaCl solution was added and the flasks agitated to mix the contents. Further solution was added to float the heptane up to the neck of the flask. Flasks were again stoppered and mixed.

(ix) Approximately 1.5 ml of the clear heptanes solution was transferred into a 4 ml vial containing approx 100 – 200 mg of anhydrous Na_2SO_4 after the phases had separated 1 ml was then transferred to a 2 ml Auto Sampler vial with a clean Pasteur pipette and the vial capped. Care was taken not to transfer particulates into the Auto Sampler vial.

3.2.21 Gas chromatography analysis of fatty acids

A Shimadzu gas chromatograph (Shimadzu Scientific Instruments, Rydalmere, New South Wales, Australia) was used with the following parameters:

- Column: J&W DB-Wax 30 m x 0.32 mm x 0.25 µm
- Injection temperature: 250°C
- Detector temperature: 285°C
- Column temperature: Start 100°C, 8°C/min to 250°C, hold 10 min, 5°C to 260°C hold 2 min.
- Carrier gas Helium at 45 kPa linear velocity 20cm/s, pressure programmed for constant flow.
- Sample injection 1 µL split ratio 15.
- Auto Sampler set for 3 sample rinses followed after injection with 3 solvent rinses.

A standard curve was calculated using the peak areas obtained for the C:17 internal standards between C:17 concentration in µg/ml and peak area. Peak area was then converted into concentration calculated from the standard curve. Internal standard correction for loss in preparation was calculated using the ratio for peak area for C:17 for each sample verses the peak area for the C:17 standard corresponding to 100 µL of standard. This ratio was used to correct each acid for loss in preparation and variation in injection volume.

3.3 Measurements

The data recorded during the study included:

Feedlot period

- Climatic data every 30 minutes using an automated weather station (Easidata Mk 4, Environdata, Warwick, Qld.) located adjacent to the feedlot in an open environment (i.e. not located under shaded pens):
  - Ambient temperature (°C)
  - Relative humidity (RH; %)
  - Wind speed (WS; m/s)
  - Wind direction
  - Solar radiation (W/m^2)
  - Black globe temperature in the sun (BG; °C)
  - Total daily rainfall (mm)

  From these data the heat load index (HLI)[1] and the accumulated heat load units (AHLU) were calculated. The relationships between the animal data (see below) and the HLI and AHLU were determined by categorizing the HLI and AHLU as follows. HLI: (1) Mild (HLI < 70), (2) Moderate (HLI 70.1 – 77), (3) Hot (HLI 77.1 – 86), (4) Very Hot (HLI 86.1 – 95) and (5) Extreme (HLI > 95). AHLU: (1) Mild (AHLU < 10), (2) Moderate (AHLU 10.1 – 25), (3) Hot (25.1 – 50), (4) Very Hot (50.1 – 100), and (5) Extreme (AHLU > 100).

[1] The HLI consists of 2 parts based on a BG threshold of 25 °C: $HLI_{BG>25} = 8.62 + (0.38 \times RH) + (1.55 \times BG) – (0.5 \times WS) + [e^{(2.4 - WS)}]$, and $HLI_{BG<25} = 10.66 + (0.28 \times RH) + (1.3 \times BG) – WS$. Where e = the base of the natural logarithm (approximate value of e = 2.71828).

The HLI and AHLU were then combined to produce 5 HLI × AHLU risk categories. HLI × AHLU: Mild: HLI<70; AHLU<10, Moderate: HLI 70.1 – 77; AHLU 10 – 20, Hot: HLI 77.1 – 86; AHLU 25 – 50, Very Hot: HLI 86.1 – 95; AHLU 50 – 100, and Extreme: HLI>95; AHLU>100.

- Non fasted (full) liveweight (kg) - pre induction on 11 September 2007, 7 October 2007 (allocation to surgery), at Induction - 11 November 2007 (Day -1) and on days 30, 60, 90, 110 and 120 (Feedlot exit). A scales accuracy check was carried out prior to each liveweight measurement occasion.
- United States Body Condition Score (USBCS) on a scale of 1 to 9 using visual assessment and palpation (Herd and Sprott 1996) pre induction on 11 September 2007, 7 October 2007 (allocation to surgery), at Induction - 11 November 2007 (Day -1) and on days 30, 60, 90, 110 and 120 (Feedlot exit).
- Visually estimated hip height in 25 mm increments pre induction on 11 September 2007, 7 October 2007 (allocation to surgery), at Induction - 11 November 2007 (Day -1) and on days 30, 60, 90, 110 and 120 (Feedlot exit).
- Individual panting score - collected daily at approximately 0600, 1200 and 1600 h every day.
- Location and posture in pen (standing or lying – in shade or sun, eating or drinking) - collected at 0600, 1200 and 1600 h every day.
- Blood collection from the 60 steers with temperature transmitters at induction (Day -1) and Day 110. Collection from a subset of 30 of the 60 steers with temperature transmitters (from the Glycerol non shade pens and from 2 replicates (both shade and non-shade pens) of the betaine treatments on days 30, 60 and 90. Blood samples were assayed for heat shock protein 70 (hsp70), blood biochemistry and haematology.
- Internal body temperature measured (between the internal abdominal muscle layer and the peritoeum at the right hand flank) in 60 steers implanted with temperature transmitters at 30 minute intervals from induction to feedlot exit, during transit to the abattoir and during abattoir lairage.
- Rectal temperature (°C) measured on the 60 steers with temperature transmitters on days 30, 60 and 110 for reconciliation with body temperatures being recorded by the temperature transmitters.
- Daily pen feed intakes on ‘as fed’ and ‘dry matter’ (DM) basis in kg/day. Daily ‘as fed’ pen intake was defined as the total feed offered per pen per day on a wet basis less any discarded residue adjusted to a similar moisture content as the original feed offered. Dry matter pen feed intake was defined as the total feed offered per pen per day on a dry matter basis as determined from the dry matter of the cumulative weekly ration samples less the dry weight of any discarded as determined by the dry matter of a sample of the residue.
- Feed conversion ratio on a DM basis in kg intake:weight gain per pen kg/day and as the inverse (weight gain per pen: kg intake).
- Assay of betaine concentration in the supplement and finisher ration.
- Analytical composition of glycerol.
- Nutritional analysis of final finisher ration on a DM basis included:
    - Crude protein (N%*6.25)
    - Metabolisable energy Mj/kg

- Total fat %
- Acid detergent fibre (ADF, %)
- Neutral detergent fibre (NDF, %)
- Ash %
- Calcium %
- Phosphorus %

- Pen water trough temperatures daily at midday (°C).
- Daily pen water usage by steers taking into account rainfall and evaporation from water troughs (L/hd/d).
- Daily observation of steer health and welfare.

During transport to abattoir following feedlot exit:

- Measurement of air temperature (°C) and relative humidity (%) at 7 locations in 4 compartments of the bottom and top decks of one 'B' Double livestock trailer.
- Internal body temperature measured (between the internal abdominal muscle layer and the peritoeum at the right hand flank) in 60 steers implanted with temperature transmitters at 30 minute intervals.
- Routine check of steer health and welfare during transit.

During abattoir lairage:

- Internal body temperature measured (between the internal abdominal muscle layer and the peritoeum at the right hand flank) in 60 steers implanted with temperature transmitters at 30 minute intervals to approximately 30 minutes pre-slaughter.

At slaughter:

- Measurement of left and right hand side carcase weights (kg).
- Overall hot standard carcase weight (HSCW) (kg).
- Dressing percentage (hot standard carcase weight (kg)/exit liveweight (kg)).
- Assessment of dentition.
- Assessment of bruising score.
- Measurement of subcutaneous fat depth (mm) at the P8 site.
- Measurement of the carcase temperature-pH profile over 3 hours post stunning.
- Placement location of each carcase in the two carcase chillers.

In the carcase chiller following 18 hours chilling at the $11^{th}/12^{th}$ quartering point on each left hand carcase side:

- Full MSA Eating Quality Grading assessment - including ossification score, eye muscle area (cm^2), rib fat thickness (mm), AUS·MEAT marbling score (range 1 to 6 and includes one-tenth gradations across the score range), numerical marbling score (range from 100 to 990 and includes 10 point gradations), AUS·MEAT meat colour score, AUS·MEAT intermuscular fat colour score, pH at 18 hours (pH_{18}) and loin temperature (°C).
- Meat texture score on a 5 point scale with 1 = coarse and 5 = fine.

- Meat firmness score on a 5 point scale with 1 = soft and 5 = firm.
- Drip loss % using a filter paper method. Score of the % wetness on the surface of filter placed on freshly cut meat.
- Surface muscle colour on the freshly bloomed muscle using a Hunter HunterLab Miniscan. Parameters included:
    - L-Hlab – muscle lightness value – 0 black and 100 white
    - a-Hlab – muscle redness-greenness value – higher values being more red, lower values being less red
    - b-Hlab – muscle yellowness-blueness value - higher values being more yellow, lower values being less yellow
- Oxymetmyoglobin (%):Metmyoglobin (%) ratio (639/580) (OM). Higher values indicating more myoglobin, lower values indicating less myoglobin.
- Cold carcase side weights (both carcase sides).

The data of Figures 20 and 21 suggest that while in motion, the microclimate within all compartments of the livestock transport was relatively stable and did not compromise steer welfare.

- Collection of 1.5 kg (200 mm long from the quartering point) of striploin for objective meat quality assay.
- Subjective hard meat score.

Objective meat tenderness analysis of the LD meat samples included:

- Moisture, dry matter and calculated chemical lean content of the lean LD (%).
- Total fat content of the lean LD (%).
- Fatty acid profile of the adipose tissue sub samples of the striploin samples (%).
- Minolta Chromameter CR300 Hunter colour space parameters of lightness ‘L’, richness of red colour ‘a’ and yellowness ‘b’.
- Sarcomere length in µm.
- Ultimate pH (pH_u).
- Cooking loss (%).
- Modified Warner Bratzler initial yield, peak force in kg and peak force minus initial yield in kg. Instron compression in kilograms was measured with a Lloyd LRX 2K5 interfaced to a computer.

3.4 Animal heat stress management

The protocol for the management of the steers during a high heat load event is described in Appendix 13.

3.5 Statistical analysis

3.5.1 General

The study was designed with pens as the experimental unit, with some observations made on individual animals, and others on the whole of pen. Wherever possible, this structure is reflected in the analysis methods, with treatment effects being evaluated against a pen level variance term rather than an animal level sampling term.

If the glycerol treatments are excluded, the remaining treatments can be considered as a randomised block design. The glycerol treatments do not fit into a block structure however, in order to sensibly analyse all of the different dietary treatments a completely randomised structure has been assumed, and any micro-environmental differences associated with pen placement have been ignored, or at least regarded as random variation. Based on the study design, a glycerol effect could be due to either glycerol *per se* or the pen position.

Many measurements were made on a repeated basis, but the bulk of the analyses were of particular times, or aggregations of observations. No attempt has been made to model sets of repeated measurements with optimal covariance structures. Instead, effort has focussed on finding intervals which can be used to allow comparisons of different measurement scales – hourly, daily, etc. There is certainly further information which can be extracted from more detailed analyses.
Data were imported into the SAS statistical program (SAS Inst. Inc., Cary, NC, USA) from the original spreadsheet files. In some cases minor editing was carried out in the spreadsheet, but the majority of data manipulation was carried out using SAS. This allowed changes to the original data to be easily tracked.

3.5.2 Weather data

3.5.2.1 Coding

Data were logged by the weather station every 30 minutes. Information was imported directly from a file produced by the weather station data-logger. Data for air temperature, relative humidity, black globe temperature, wind speed and heat load index were summarised to a daily basis by calculating the mean, minimum and maximum for each 24 hour period (midnight to midnight). For solar radiation readings, night time (zero) values were first excluded, and the mean and maximum values calculated for each day on the remaining data. Rainfall was totalled for each day. The accumulated heat load (AHLU) was retained in its original form (Gaughan *et al.* 2008a).

The AHLU was examined in order locate periods of likely heat stress. The period from 16 January to 19 February appeared interesting as it commenced with 4 days where the AHLU was low (<10), followed by 22 days where the AHLU generally classified as high or above (>25), and concluding with 9 days where the AHLU was low.

3.5.3 Pen level measurements

3.5.3.1 Coding

Data recorded at the pen level on a daily basis combined the information on dry matter and water intake, and on water temperature. These data were all recorded on a daily basis throughout the trial. Average daily figures were calculated for each of the four monthly intervals for each pen. In addition cumulative average values from the start of the trial were calculated to the end of each ‘trial month’.

3.5.3.2 Analysis

Pen measurement data were analysed using the GLM procedure, with terms for diet, shade and their interaction. Least squares means were estimated for the various treatment effects, with pair-wise testing carried out when an effect was significant ($P<0.05$) using a least significant difference (LSD). In addition, estimates and standard errors were derived for the average change due to shade, the average effect of glycerol versus control and the average effect of betaine versus control.

For the identified hot period, the daily intake data were analysed in exactly the same fashion as the monthly averages.

3.5.4 Live weight and growth data

3.5.4.1 Coding

These data consisting of weights, condition scores and hip heights, all originated from the 'Master file' data set, and limited modification was necessary prior to analysis. In addition to the growth intervals based upon the 30 day weighing dates, further intervals covering 10 February to 1 March, 2008 and 1 March to 11 March, 2008 were calculated. Average per pen growth figures were also calculated for each of the intervals being examined. These values were then combined with the intake data described in the previous section to produce feed conversion ratios.

3.5.4.2 Analysis

Analysis of variance models were fitted with the MIXED procedure, using REML estimation. Tests of diet and shade effects and their interaction were evaluated against the between pen variance. At the animal level, the trial allocation group was included as a covariate, as well as initial weight (for live-weight and weight gain), initial hip height (for hip-height and changes in hip-height) and initial condition score (for body condition scores). Least squares means were estimated for the various treatment effects, with pair-wise testing carried out when an effect was significant ($P<0.05$). For presentation purposes, a weighted average least significant difference (LSD) was derived which took account of differing numbers in each pen. In addition, estimates and standard errors were derived for the average change due to shade, the average effect of glycerol versus control, the average effect of betaine versus control, and the average effect of a surgical implant.

Data on feed conversion ratios were calculated at a pen level and were analysed using the GLM procedure with terms for diet, shade and their interaction. Least squares means were estimated for the various treatment effects, with pair-wise testing carried out when an effect was significant ($p<0.05$) using a least significant difference (LSD). In addition, estimates and standard errors were derived for the average change due to shade, the average effect of glycerol versus control and the average effect of betaine versus control.

3.5.5 Body temperature data

3.5.5.1 Coding

All valid observations were combined into a single data set with more than 250,000 records. Each observation was identified by an animal number and actual observation time (different for each transmitter), as well as an 'adjusted' time, being the nearest half hour (common to all animals). Observations were further identified as being collected while the animals were in pens, being

transported, or in lairage prior to processing. During livestock transport, animals were identified as travelling on either the upper or lower deck of the truck.

For each animal on each day, the average temperature reading, and the temperature range (maximum – minimum) were derived. Any day where an animal had less than 24 valid readings logged was then removed. This daily summary was further processed so that for each animal for each of the 4 month periods during the trial, the average of the average daily temperature and the average range in daily temperature was derived for each animal.

3.5.5.2 Analysis

For the data summarised by month, analysis of variance models were fitted with the MIXED procedure, using REML estimation. Tests of diet and shade effects and their interaction were evaluated against the between pen variance. At the animal level, only the trial allocation group was included as a covariate. Least squares means were estimated for the various treatment effects, with pair-wise testing carried out when an effect was significant ($P<0.05$). For presentation purposes, a weighted average least significant difference (LSD) was derived which took account of differing numbers in each pen. In addition, estimates and standard errors were derived for the average change due to shade, the average effect of glycerol versus control, and the average effect of betaine versus control.

For each day in the identified hot period, the minimum, maximum and mean body temperature was calculated, as well as the daily range in body temperature. A separate mixed model analyses was carried out on each day's data as outlined in the previous paragraph.

Detailed analysis took place of the final days of the trial, including transport and pre-slaughter periods. From 0800 h on 10 March 2008 until 0830 h on 11 March 2008, the animals were analysed as outlined above. It should be noted that the number of animals transmitting data at any one time varied depending on their proximity to the receiver.

From 1030 h until 1700 h on 11 March 2008, the animals were in transit to the abattoir. A similar mixed model analysis was used, with the addition of deck as covariate. In addition to the treatment means already described, least squares means and standard errors for each of the decks were estimated.

Finally, from 1800 h on 11 March until 0900 h on 12 March 2008 animals were in lairage at the abattoir. The statistical model was the same as that used when the animals were in the feedlot, although technically all were housed together. The pen grouping were retained however to test for any carry over effects of the treatments into the next day.

3.5.6 Panting score data

3.5.6.1 Coding

Panting score was recorded on a 0 to 4.5 scale. The scale used had been designed to behave in a reasonably linear fashion, so for each of the monthly intervals, the mean panting score was calculated for each animal. To better account for the bounded nature of the scale, the same calculations were carried out using the angular transformed panting score.

For the identified hot period, at a daily level, the mean panting score for each pen was calculated.

3.5.6.2 Analysis

For the data summarised by month, analysis of variance models were fitted with the MIXED procedure, using REML estimation. Tests of diet and shade effects and their interaction were evaluated against the between pen variance. At the animal level, only the trial allocation group was included as a covariate. Residual diagnostics were examined and the results for both the simple average panting score and the average angular transformed panting score were compared. Although offering a slight improvement for some of the analyses of the early morning and late afternoon scores, the differences in results were very minor. Accordingly, only results for the simple average panting score are presented.

Least squares means were estimated for the various treatment effects, with pair-wise testing carried out when an effect was significant ($P<0.05$). For presentation purposes, a weighted average least significant difference (LSD) was derived which took account of differing numbers in each pen. In addition, estimates and standard errors were derived for the average change due to shade, the average effect of glycerol versus control, the average effect of betaine versus control, and the average effect of a surgical implant.

For the identified hot period, individual animals were not considered, only the pen average. Initially a generalised linear model was used on both the original score data and on a modified scale where an animal was classified as stressed ($PS \geq 2$), or not stressed ($PS < 2$). This approach was only possible on days when some stress was present. It was found that on some days no animals showed any stress, and others where all animals showed stress. Using the average per pen panting score, weighted according to the number of animals in the pen and analysed using a simple general linear model, we were able to process all days in a consistent manner. Only terms for diet, shade and their interaction were included and least squares means and standard errors estimated for each of the treatment effects.

3.5.7 Blood assay data

Limitations within the full dataset prevented the fitting of a three-way interaction for diet x shade x month, however the three-way interaction was able to be included using a sub-set of the full data where January and glycerol data was omitted. This analysis demonstrated that the three-way interaction of diet x shade x month was not significant when fitted on the sub-set data. It was therefore inferred that this interaction could be omitted from analysis of the full data-set (January and Glycerol treatments included) without loss of information.

Blood parameters were analysed using repeated measures in PROC MIXED. Results were analysed for month x shade and month x diet effects for all measurements, and included fixed effects for month (November, December, January, February and March), diet (Control, Betaine 10, Betaine 20, Betaine 40 and Glycerol), shade (Nil Shade and Shade), diet x month, shade x month and diet x shade. The model also included random effects for replication. Pen was the experimental unit. Where effects were significant, Pair-Wise comparisons of the least squares means were carried out within each month. For presentation purposes, a pooled LSD was calculated for comparisons within each month.

3.5.8 Slaughter data

3.5.8.1 Coding

This data set combines information from ‘Abattoir Carcase Data’, ‘MSA Grade data’, ‘FSA data’ and ‘RW colour data’. Data were matched on the basis of the Brigalow ID tag, giving 164 cases. The

position of each body/carcase when the Abattoir slaughter floor chain broke down was imported for use as a covariate.

A number of variables were modified prior to analysis or eliminated altogether.

- The MSA meat colour data (MC) was coded as either 1B (n=117), 1C (n=43) or 2 (n=4). This was converted to a simple 0/1 variable based on 1B=0, anything else=1
- The EPBI was ignored since all data were zero
- Dentition was ignored as all except 3 animals had dentition=0
- Bruising was ignored as all right hand carcase sides were zero, and only one animal showed any left hand carcase side bruising
- The Abattoir Grade Scores (SF or G) for the two carcase sides were identical for the 164 trial animals, so were only included once. The score was recoded so that a grade of G was 0 and a grade of SF was 1
- Meat hardness was identical for the two carcase sides, and was recoded so that 'normal' meat was coded zero, and 'hard' meat coded as 1
- The abattoir personnel grading of meat colour (MC) and fat colour (FC) were ignored since all samples were the same (1C and 0 respectively).

3.5.8.2 Analysis

Analysis of variance models were fitted with the MIXED procedure, using REML estimation. Tests of diet and shade effects and their interaction were evaluated against the between pen variance. Various individual animal level covariates were included.

The analysis of the abattoir data was complicated by the slaughter floor chain breakdown that occurred. Since animals with temperature transmitters were processed first, the breakdown created quite severe confounding. The majority of the animals with transmitters (63%) had been fully processed and were in the carcase chiller when the breakdown occurred, with the balance being partially processed. On the other hand, the non-surgical animals were split between being partially processed (74%) and not yet stunned (26%). In order to deal with this unfortunate pattern, the position on the processing line was included as a linear covariate. Also included were a 0/1 covariate to represent the surgical implant, and the pre-trial liveweight. Trial allocation group was unable to be included as a covariate since it was based in part on the surgical modification, and was thus partly confounded with position on the processing line. The model used essentially allows for an approximately linear relationship between the variable being analysed and the processing order. This response is then offset by varying amounts depending upon treatment and surgery.

Least squares means were estimated for the various treatment effects, with pair-wise testing carried out when an effect was significant ($P<0.05$). For presentation purposes, a weighted average least significant difference (LSD) was derived which took account of differing numbers in each pen. In addition, estimates and standard errors were derived for the average change due to shade, the average effect of glycerol versus control, the average effect of betaine versus control, and the average effect of a surgical implant.

3.5.8.3 Adipose tissue fatty acid profile data

Fatty acid composition data was analysed using repeated measures in PROC GLM. The model for all measurements included terms for diet (Control, Betaine 10, Betaine 20 and Betaine 40 (Nil Shade and Shade), and diet x shade interaction. Since only one pen of each treatment was sampled, the individual animal was used as the experimental unit. Where the *F*-test was significant, Pair-Wise differences were conducted on the least squares means using t-tests. Treatment effects were tested against a between animal error term.

4 Results and discussion

4.1 Weather conditions

Overall, the study period was cooler on average, with some intermittent hot days over 35 °C and average rainfall recorded. Rainfall, minimum, mean, maximum wind speed and relative humidity for the study are shown in Figure 1. When compared to the 40 year mean monthly rainfall for BRS, December rainfall was average, January above average and February slightly below average.

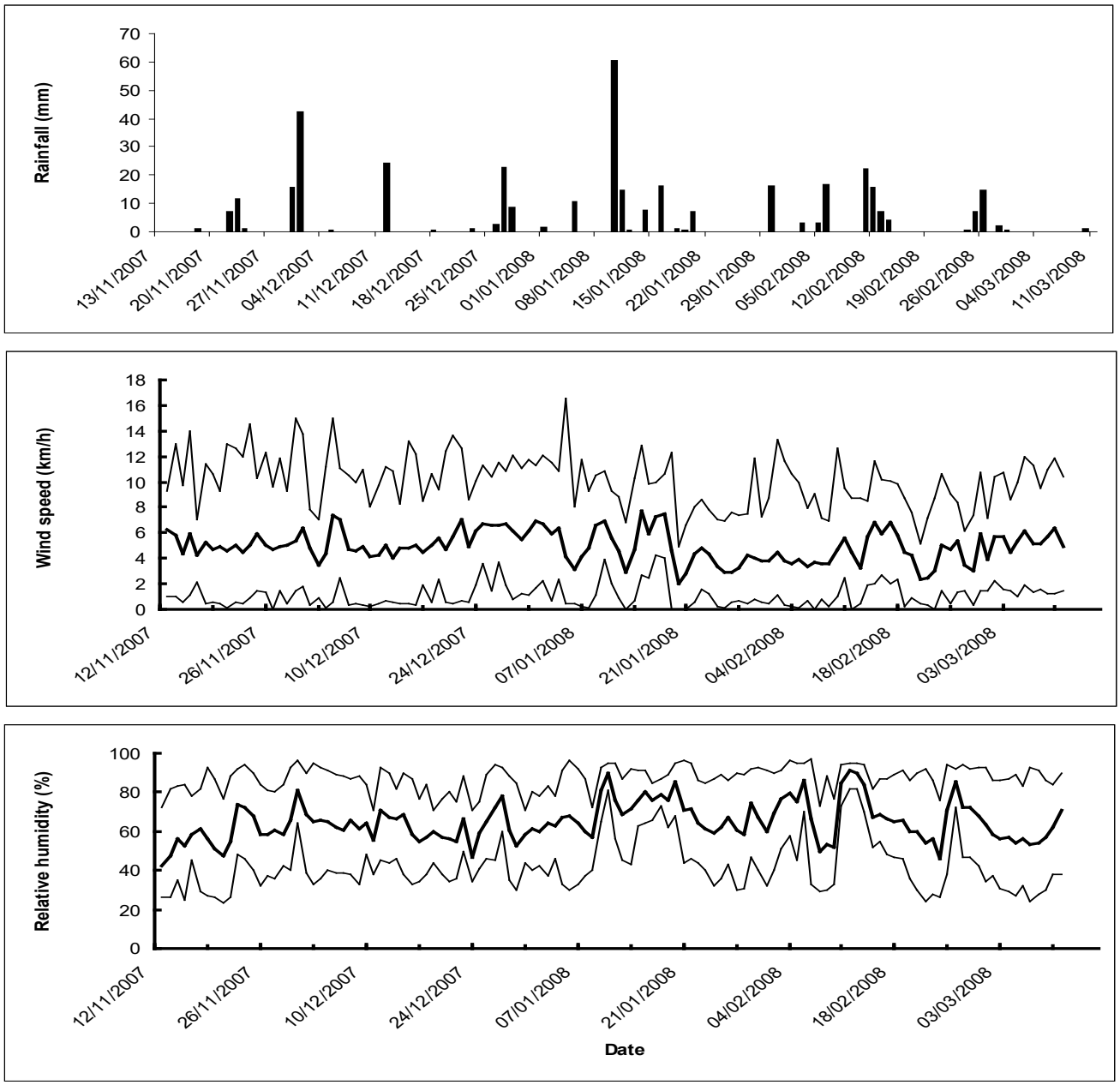

Figure 1 Rainfall, minimum, mean, maximum wind speed and relative humidity over the study period

Air temperature, black globe temperature and wind speed are shown in Figure 2. The highest ambient temperature recorded in November was 34.2°C, in December 35.1°C, in January 36.7°C, in February 38.7°C and in March, 31.3°C. The maximum ambient temperature exceeded 30°C on 74 days, however only exceeded 35°C on 6 days.

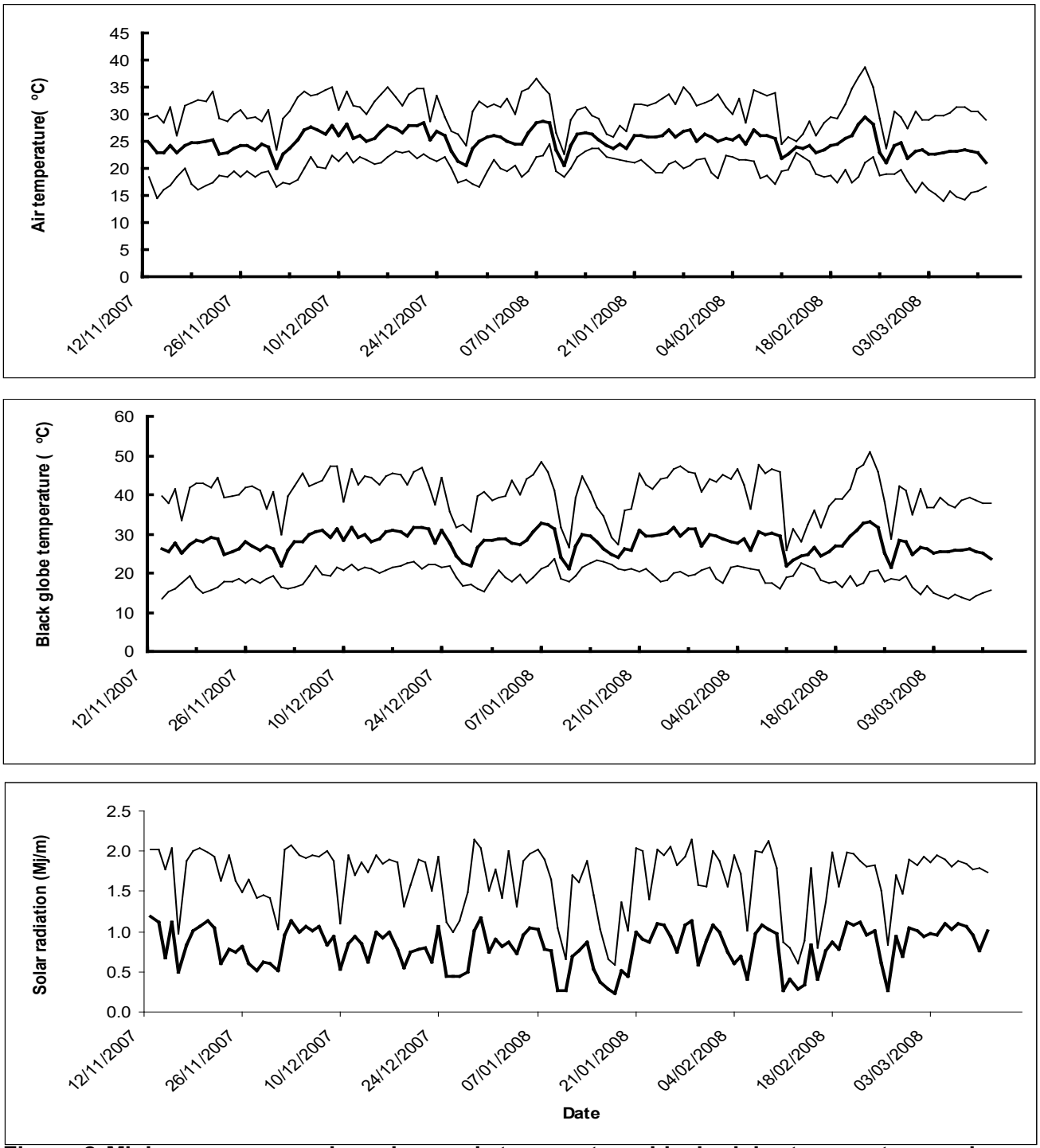

Figure 2 Minimum, mean and maximum air temperature, black globe temperature and mean and maximum wind speed over the study period

The minimum, mean and maximum HLI calculated for the study period is shown in Figure 3. There were 89 days with a maximum HLI > 86, 60 days were HLI > 90, 39 days with a HLI > 95 and 14 days when HLI >100. The maximum HLI recorded was 105.7 (1200 h, February 4, 2008). The maximum HLI were sufficient to induce heat stress in the un-shaded cattle for at least 89 days of the study. However throughout most of the period there was sufficient night-time cooling to allow the cattle to dissipate accumulated body heat back to the environment. The HLI < 60 on 93 nights, and was less than 55 on 32 of these nights.

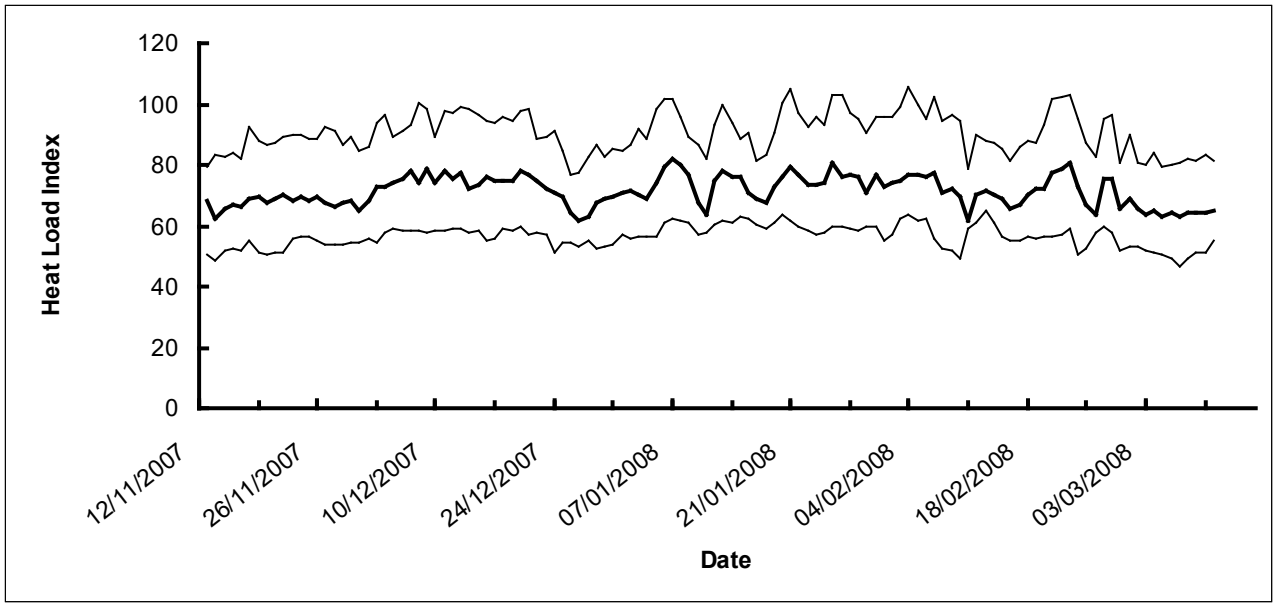

Figure 3 Minimum, mean and maximum Heat Load Index calculated during the study period

The accumulated heat load units (AHLU) calculated during the study period are presented in Figure 4. Heat load events (AHLU >50) occurred in December 2007, January and February 2008. The heat load events were identified during a 34 day period between Days 65 (16 January, 2008 and 99 (19 February 2008) of the feeding period. During this period, AHLU values up to 106 were recorded. However values greater of 100 were only recorded on two days (January 7, 2008 and January 26, 2008). The most prolonged period of high AHLU values were recorded from January 20 to February 10, 2008.

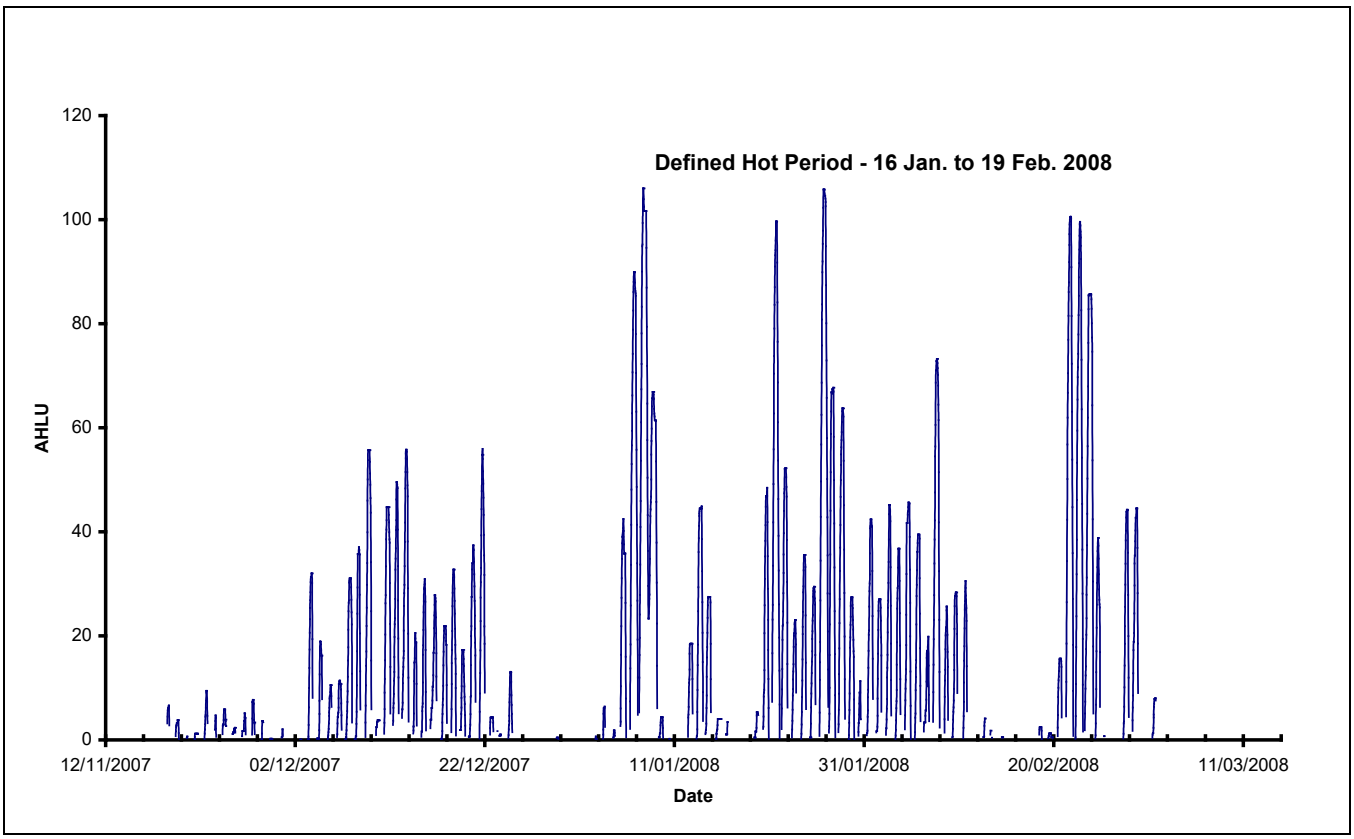

Figure 4 Study period Accumulated Heat Load Units

4.2 Animal health

There were minimal health issues associated with the steers for the period they were on Brigalow Research Station, despite their relocation from Central NSW to Central Queensland. The steers adapted well to the climatic conditions in Central Queensland.

Following arrival and prior to 9 October 2007, 17 steers (10%) were treated with either 3 g Opticlox™ Eye Ointment (835 mg cloxacillin as benzathine salt, 5 g cloxacillin, Norbrook Laboratories Limited, Northern Ireland) or 3 g Orbenin® Eye Ointment (500 mg cloxacillin as benzathine salt, Pfizer Australia Pty Ltd) for bovine infectious keratoconjunctivitis (BIK). Over the post-surgery period from 13 October, 2007 until 26 November, 2007 28 steers (16%) were also treated as required with Orbenin®Eye Ointment (500 mg cloxacillin as benzathine salt, Pfizer Australia Pty Ltd) for BIK. During the pre and post-surgery periods, a number of the steers were re-treated. No further treatment for BIK was required after 26 November, 2007.

During the overall study period, 5 steers (2.8%) were treated with 50 ml/100kg liveweight Engemycin™ 100 (oxytetracycline hydrochloride 100 mg/ml injection, Intervet Australia Pty Ltd) and 3 ml/100 kg liveweight Key Injection (ketoprofen 100 mg/ml, Parnell Laboratories Australia Pty Ltd) for infections to their feet. These infections were generally a consequence of trauma and occurred prior to feedlot entry except for two steers.

Four steers (2%) were treated with 2.5 ml/100 kg liveweight Imidox Injection (Imidocarb dipropionate 120 mg/ml, Parnell Laboratories (AUST) Pty Ltd) on 20 October 2007 for a suspected tick fever

(anaplasmosis) reaction. The steers had been vaccinated with a trivalent tick fever vaccination (mixed bovine blood containing *Babesia bovis* & *Anaplasma centrale & Babesia bigemina,* DPI Tick fever Centre, Wacol Queensland) and treated with Cydectin® Pour On (5 g/l moxidectin solvent, 150 g/l hydrocarbon liquid, Fort Dodge Australia Pty Ltd) upon arrival at Brigalow Research Station.

Of the 63 steers surgically implanted with temperature transmitters on 9 and 10 October, 2007, only 3 (4%) had slightly gaping post-operative wounds at the point of incision and were subsequently treated with 50 ml/100kg liveweight Engemycin™ 100 (oxytetracycline hydrochloride 100 mg/ml injection, Intervet Australia Pty Ltd) on one occasion only.

During the feedlot period, there was no visual evidence of any digestive issues – acidosis or bloat.

4.3 Feed analysis

The nutritional analysis of the study diets is shown in Table 7. The crude protein level of each treatment diet was higher than the theoretical levels of 13.2 to 13.3%). The higher than expected protein was a consequent of the high wheat protein content. All other diet ingredient protein levels were within the expected range. The crude protein values were similar across all diets, while the total fat was higher for the Glycerol treatment. Acid detergent fibre and neutral detergent fibre values tended to vary across treatments. Ash values were similar across treatments, while the Control treatment calcium levels were higher than other treatments. Phosphorus levels were similar for each treatment. Overall, both calcium and phosphorus levels were lower than the theoretical levels (Ca of 0.80 to 0.83% and P of 0.40 to 0.42%).

Table 7 Approximate analysis of the diets used

	Dry matter (%)	Crude protein (%)	Est. ME (Mj/Kg)	Total fat (%)	Acid detergent fibre[2] (%)	Neutral detergent fibre[2] (%)	Ash (%)	Ca (%)	P (%)
Control	84.0	16.9	13.0	5.0	10.7	22.7	4.9	0.78	0.30
Betaine 10	84.1	16.8	12.9	4.7	12.6	24.0	4.8	0.74	0.31
Betaine 20	84.0	16.8	13.1	4.7	9.8	21.1	4.9	0.73	0.30
Betaine 40	83.9	16.9	13.1	5.2	10.6	23.3	5.0	0.68	0.30
Glycerol	83.2	16.7	13.3	6.9	11.8	23.1	4.7	0.66	0.30

[1]ME = Metabolisable energy. [2]ADF = acid detergent fibre (ash free). [2]NDF = neutral detergent fibre (ash free).

4.4 Supplement analysis

The analysed concentration of betaine in the respective treatment supplements and the theoretical concentrations are shown in Table 8. There was an inherent background concentration of betaine in the Control betaine placebo, Control glycerol placebo, Control/betaine base and glycerol base supplements as a consequence of the grain cereal carrier that comprised a significant proportion of the composition of the supplements (Tables 4 and 5). The analysed betaine concentrations are slightly lower than the calculated theoretical betaine concentrations for the Betaine 10, Betaine 20 and Betaine 40 supplements.

Table 8 Concentration of betaine (mg/g) in the treatment supplements on a fresh weight basis

Supplement	Analysed betaine concentration	Theoretical betaine concentration
Control betaine placebo	5.46	0.00
Control glycerol placebo	5.37	0.00
Betaine 10	55.83	67.30
Betaine 20	101.97	134.70
Betaine 40	213.37	270.00
Control/betaine base	2.35	0.00
Glycerol base	1.20	0.00

4.5 Analytical composition of glycerol

The chemical composition of the glycerol fed in the Glycerol treatment is shown in Table 9. Batches 1, 2 and 3 were fed sequentially over the study period. While the 3 separate batches came from the same initial consignment, there is little difference in any analyte apart from sulphur concentration between batches. The glycerol level in each batch was desirable for animal feeding and importantly both methanol and the matter organic non glycerol (MONG) values were low and would not have affected animal acceptability of the glycerol inclusion. The glycerol inclusion rate was 5% of the ration on an as fed basis for the finisher diet.

Table 9 Chemical composition of glycerol fed during study (as fed basis)

Batch	Moisture (%w/w)	Solids (%w/w)	Ash (%w/w)	Glycerol (%w/w)	Methanol (%w/w)	Matter organic non glycerol (%w/w)	Potassium (mg/kg)	Sulphur (mg/kg)	Sodium (mg/kg)
1	24.9	75.1	4.2	71.6	0.04	<0.1	15,800	22,000	400
2	25.0	75.0	4.2	71.4	0.04	<0.1	16,900	24,000	410
3	24.7	75.3	4.1	72.0	0.04	<0.1	16,700	23,200	410

4.6 Feed intakes

Dry matter feed intake (DMI) was not affected ($P>0.05$) by dietary treatment (Table 10). Access to shade had a positive response on DMI. Cattle in shaded pens, irrespective of diet had a greater ($P<0.05$) DMI from day 30 to the end of the study. For the overall study period, cattle in shaded pens recorded a greater ($P<0.05$) DMI. There were no diet × shade interactions.

Table 10 Effect of diet (Betaine 10 (10 g/steer/d), 20 (20 g/steer/d) or 40 (40 g/steer/d)) or glycerol (5% DMB) and shade (no shade and shade) and their interaction on dry matter feed intake from induction (I) to exit (E) at 120 days on feed

	Dry matter feed intake (kg/hd/d)						
	I-30d	30d-60d	I-60d	60d-90d	I-90d	90d-120d	I-E
Diet (D)							
Control	8.8[A]	10.2	9.5	10.5	9.8	10.4	10.0
Betaine 10	8.9	10.6	9.8	10.7	10.1	10.6	10.2
Betaine 20	9.1	10.6	9.9	10.8	10.2	10.6	10.3
Betaine 40	8.9	10.3	9.6	10.5	9.9	10.4	10.0
Glycerol	9.1	10.3	9.7	10.7	10.1	10.6	10.2
SE	0.2	0.2	0.2	0.1	0.2	0.1	0.2
Shade (S)							
Nil Shade	8.8	10.1[b]	9.5[b]	10.5[b]	9.8[b]	10.4[b]	10.0[b]
Shade	9.1	10.7[a]	9.9[a]	10.8[a]	10.2[a]	10.7[a]	10.3[a]
SE	0.1	0.1	0.1	0.1	0.1	0.1	0.1
D x S							
Control-Nil Shade	8.9	10.1	9.5	10.5	9.8	10.1	9.9
Control-Shade	8.7	10.3	9.5	10.5	9.8	10.7	10.0
Betaine 10-Nil Shade	8.6	10.3	9.4	10.5	9.8	10.5	9.9
Betaine 10-Shade	9.3	11.0	10.2	11.0	10.4	10.7	10.5
Betaine 20-Nil Shade	8.9	10.1	9.5	10.4	9.8	10.3	9.9
Betaine 20-Shade	9.4	11.0	10.2	11.2	10.5	10.8	10.6
Betaine 40-Nil Shade	9.0	10.2	9.6	10.4	9.9	10.1	9.9
Betaine 40-Shade	8.8	10.5	9.7	10.6	10.0	10.7	10.2
Glycerol-Nil Shade	8.8	10.1	9.4	10.7	9.8	10.7	10.1
Glycerol-Shade	9.5	10.6	10.0	10.7	10.3	10.6	10.3
SE	0.3	0.3	0.3	0.2	0.2	0.2	0.2

[A] I – 30d = DMI from induction (I) to day 30; 30d – 60d = ADG from day 30 to day 60, and so on.
Means within a column within an effect with different superscripts are significantly different ($P < 0.05$). SE = standard error, d = days

The mean daily steer intake of betaine for each treatment is presented in Table 11. The results indicate that mean daily betaine intakes were slightly below (Betaine 10) or below (Betaine 20 and Betaine 40) the desired intakes. The assumptions on which the mean betaine intakes are based on are: the betaine supplements were well dispersed through the feed mixture, that all steers within a pen always had equal access to the feed bunks and that all ration was eaten each day prior to feeding (as per The Clean Bunk at Midday program). On average, the steers in both the non-betaine treatments (Control and Glycerol) consumed approximately 1 g/hd/d of betaine. As indicated in Section 4.4, this background level of betaine was a consequence of the grain cereal carrier that comprised a significant proportion of the composition of the supplements.

Table 11 Mean daily steer intake of betaine across diets (g/hd/d)

Diet	Daily betaine intake from treatment supplement	Background betaine intake from base supplements and placebo supplements	Total daily betaine intake
Control	0.00	1.18	1.18
Betaine 10	8.72	0.33	9.05
Betaine 20	15.93	0.33	16.26
Betaine 40	33.34	0.33	33.67
Glycerol	0.00	0.94	0.94

4.7 Water consumption

Water temperature

Daily water temperatures were higher over days 30 to 60 in the Glycerol pens ($P<0.01$) compared to the control or betaine treatment pens (Table 12). There were no differences in water temperatures ($P>0.05$) between the dietary treatment pens for the other intervals nor overall. Shaded pens recorded higher water temperatures for days 30 to 60 ($P<0.01$) and days 60 to 90 ($P<0.05$). The diet × shade interaction data indicates there was no difference in water temperature due to shade in the glycerol pens for days 30 to 60. For days 30 to 60 and days 60 to 90 the diet × shade interaction means suggest that shaded pens recorded higher water temperatures but not significantly higher ($P>0.05$). There is no apparent reason for the higher temperatures of the Glycerol treatment pens other than those pens were closer to the feedlot water storage tanks. All water troughs and underground water pipes were of similar specifications. All water troughs were located outside of the shade structure, however as indicated, water temperatures in the shaded treatment pens were higher on some occasions.

While the water temperatures were higher in the shaded pens for days 30 to 60 and days 60 to 90, these higher temperatures had no effect on DMI in the same pens for the corresponding period.

Table 12 Water temperatures recorded in treatment pens

	Pen water temperature (°C)				
	I-30d[A]	30d-60d	60d-90d	90d-120d	I-E
Diet (D)					
Control	31.37	30.56[b]	30.81	28.76	30.23
Betaine 10	31.40	30.61[b]	30.85	28.83	30.28
Betaine 20	31.44	30.69[b]	30.87	28.91	30.34
Betaine 40	31.52	30.78[b]	30.95	28.92	30.40
Glycerol	31.95	31.63[a]	31.77	29.69	31.16
SE	0.26	0.15	0.27	0.84	0.30
Shade (S)					
Nil Shade	31.40	30.64[b]	30.78[b]	28.85	30.28
Shade	31.67	31.07[a]	31.32[a]	29.18	30.69
SE	0.16	0.09	0.17	0.53	0.19
D x S					
Control-Nil Shade	31.35	30.38	30.62	28.60	30.08
Control-Shade	31.39	30.74	31.00	28.92	30.39
Betaine 10-Nil Shade	31.18	30.38	30.52	28.65	30.04
Betaine 10-Shade	31.62	30.84	31.18	29.00	30.52
Betaine 20-Nil Shade	31.24	30.45	30.63	28.76	30.13
Betaine 20-Shade	31.64	30.93	31.11	29.06	30.55
Betaine 40-Nil Shade	31.35	30.63	30.64	28.75	30.20
Betaine 40-Shade	31.69	30.93	31.26	29.08	30.60
Glycerol-Nil Shade	31.87	31.36	31.48	29.51	30.94
Glycerol-Shade	32.02	31.89	32.05	29.87	31.38
SE	0.37	0.21	0.38	1.19	0.42

[A] I – 30d = water temperature from induction (I) to day 30; 30d – 60d = ADG from day 30 to day 60, and so on.
Means within a column within an effect with different superscripts are significantly different (*P < 0.05*). SE = standard error, d = days

Water consumption

Dietary treatment did not have an effect (P>0.05) on water consumption (Table 13). However there was a shade effect (P<0.01) between days 60 – 90 days (P<0.001) and 90 – 120 days (P<0.01) on feed where water consumption was greater in the un-shaded pens. These two periods corresponded with higher heat load over a 34 day defined hot period as indicated by the AHLU data of Figure 4. There were no diet × shade interactions.

Of interest is that steers with shade while consuming less water over days 60 to 120, at the same time recorded a higher DMI. In addition, higher water temperatures of the shaded pens (P<0.05) over days 60 to 90 (Table 12) coincided with the lower water consumption (P<0.05) of these pens over the same period.

Table 13 Effect of diet (Betaine 10 (10 g/steer/d), 20 (20 g/steer/d) or 40 (40 g/steer/d)) or glycerol (5% DMB) and shade (no shade and shade) and their interaction on pen water consumption

	Water consumption (L/hd/d)						
	I-30d	30d-60d	I-60d	60d-90d	I-90d	90d-120d	I-E
Diet (D)							
Control	35.9[A]	50.1	43.0	54.5	46.8	48.0	47.1
Betaine 10	40.6	59.5	50.0	58.1	52.7	51.2	52.3
Betaine 20	46.0	64.3	55.2	59.3	56.5	52.9	55.6
Betaine 40	39.9	53.6	46.8	51.9	48.5	46.5	48.0
Glycerol	44.0	61.9	53.0	56.3	54.1	49.6	53.0
SE	4.7	4.4	4.0	2.0	2.9	1.7	2.4
Shade (S)							
Nil Shade	39.3	59.6	49.4	60.7[a]	53.2	53.1[a]	53.1
Shade	43.3	56.2	49.8	51.3[b]	50.3	46.2[b]	49.3
SE	3.0	2.8	2.5	1.2	1.8	1.1	1.5
D x S							
Control-Nil Shade	31.1	57.2	44.2	61.1	49.8	52.1	50.4
Control-Shade	40.8	42.9	41.8	48.0	43.9	43.9	43.9
Betaine 10-Nil Shade	34.0	56.8	45.4	62.1	51.0	55.9	52.2
Betaine 10-Shade	47.2	62.2	54.7	54.0	54.4	46.6	52.5
Betaine 20-Nil Shade	54.7	65.3	60.0	64.1	61.4	55.1	59.8
Betaine 20-Shade	37.4	63.4	50.4	54.4	51.7	50.7	51.5
Betaine 40-Nil Shade	36.9	52.9	44.9	53.7	47.9	46.0	47.4
Betaine 40-Shade	42.9	54.3	48.6	50.1	49.1	47.0	48.6
Glycerol-Nil Shade	39.6	65.5	52.5	62.3	55.8	56.2	55.9
Glycerol-Shade	48.5	58.3	53.4	50.2	52.4	43.0	50.0
SE	6.7	6.3	5.6	2.8	4.0	2.5	3.4

[A] I – 30d = water consumption from induction (I) to day 30; 30d – 60d = ADG from day 30 to day 60, and so on.
Means within a column within an effect with different superscripts are significantly different ($P < 0.05$). SE = standard error, d = days

4.8 Change in liveweight

Dietary treatment did not have an effect (P>0.05) on liveweight on any date from induction to exit (Table 14). The Glycerol treatment Exit liveweights were numerically similar to the Control and lower than the Betaine treatments. However, the provision of shade resulted in heavier cattle on days 30 (P<0.05), 60 (P<0.01), 90 (P<0.05), 110 (P<0.01) and on exit (P<0.001). There were no diet × shade interactions. Exit liveweights met target liveweights suitable for the 'short-fed' export grain-fed beef market.

Table 14 Effect of diet (Betaine 10 (10 g/steer/d), 20 (20 g/steer/d) or 40 (40 g/steer/d)) or glycerol (5% DMB) and shade (no shade and shade) and their interaction on steer liveweight from induction to exit (120 days)

	Liveweight (kg)					
	Induction (I)	Day 30 (30d)	Day 60 (60d)	Day 90 (90d)	Day 110 (110d)	Exit (E)
Diet (D)						
Control	392.8	438.7	493.5	536.2	560.9	583.9
Betaine 10	399.2	448.1	502.6	543.7	570.3	591.5
Betaine 20	398.2	448.5	505.6	544.4	571.5	593.1
Betaine 40	399.2	444.4	499.1	539.4	565.4	586.2
Glycerol	395.5	442.2	498.4	538.5	562.1	582.1
SE	3.5	3.6	3.5	3.4	4.5	3.8
Shade (S)						
Nil Shade	396.0	439.9[b]	494.0[b]	535.7[b]	559.3[b]	578.6[b]
Shade	398.0	448.9[a]	505.7[a]	545.1[a]	572.8[a]	596.1[a]
SE	2.3	2.3	2.3	2.2	2.9	2.4
D x S						
Control-Nil Shade	392.2	436.9	493.1	533.8	556.0	578.9
Control-Shade	393.4	440.4	493.9	538.6	565.8	588.9
Betaine 10-Nil Shade	402.7	438.2	494.4	536.7	562.3	580.2
Betaine 10-Shade	395.7	457.9	510.9	550.7	578.2	602.7
Betaine 20-Nil Shade	397.3	443.3	495.1	535.8	559.6	580.3
Betaine 20-Shade	399.0	453.8	516.1	552.9	583.5	605.9
Betaine 40-Nil Shade	397.7	442.1	495.9	537.1	558.8	579.5
Betaine 40-Shade	400.8	446.7	502.3	541.6	572.1	592.9
Glycerol-Nil Shade	390.0	438.8	491.7	535.3	559.9	574.1
Glycerol-Shade	401.0	445.6	505.1	541.7	564.3	590.1
SE	5.0	5.1	5.0	4.8	6.3	5.3

Means within a column within an effect with different superscripts are significantly different ($P < 0.05$). SE = standard error, d = days

Dietary treatment had no effect (P>0.05) on average daily gain throughout the study period (Table 15). The Glycerol treatment overall (I-E) average daily gain was numerically lower than all other treatments. The provision of shade increased average daily gain over every interval of the study period except for days 30 to 60 and 60 – 90; with I – 30 (P<0.05), I – 60 (P<0.01) I – 90 ((P<0.05), 90 – 110 (P<0.05), I – 110 (P<0.01), 90 – E (P<0.01) and I – E (P<0.001). There were no diet × shade interactions. The magnitude of the average daily gains were acceptable given that unadapted *Bos taurus* steers without hormonal growth promotants were being fed over the summer period in Central Queensland.

Table 15 Effect of diet (Betaine 10 (10 g/steer/d), 20 (20 g/steer/d) or 40 (40 g/steer/d)) or glycerol (5% DMB) and shade (no shade and shade) and their interaction on average daily gain (ADG) from induction (I) to exit (120 days)

	ADG (kg/hd/d)								
	I-30d	30d-60d	I-60d	60d-90d	I-90d	90d-110d	I-110d	90d-E	I-E
Diet (D)									
Control	1.374[A]	1.828	1.597	1.424	1.540	1.195	1.484	1.590	1.552
Betaine 10	1.678	1.819	1.747	1.368	1.622	1.323	1.568	1.592	1.615
Betaine 20	1.692	1.902	1.796	1.291	1.630	1.353	1.579	1.625	1.628
Betaine 40	1.560	1.823	1.689	1.341	1.575	1.298	1.524	1.562	1.571
Glycerol	1.488	1.874	1.678	1.335	1.565	1.175	1.494	1.455	1.538
SE	0.117	0.089	0.058	0.076	0.038	0.097	0.041	0.075	0.031
Shade (S)									
Nil Shade	1.413[b]	1.805	1.606[b]	1.390[a]	1.535[b]	1.159[b]	1.469[b]	1.430[b]	1.509[b]
Shade	1.704[a]	1.893	1.797[a]	1.314[b]	1.638[a]	1.378[a]	1.590[a]	1.700[a]	1.653[a]
SE	0.074	0.056	0.037	0.049	0.024	0.062	0.026	0.048	0.020
D x S									
Control-Nil Shade	1.318	1.872	1.590	1.357	1.513	1.035	1.440	1.504	1.511
Control-Shade	1.430	1.783	1.603	1.491	1.566	1.355	1.527	1.676	1.593
Betaine 10-Nil Shade	1.360	1.871	1.612	1.410	1.546	1.273	1.496	1.451	1.522
Betaine 10-Shade	1.995	1.766	1.882	1.325	1.699	1.373	1.640	1.734	1.707
Betaine 20-Nil Shade	1.523	1.727	1.623	1.357	1.536	1.184	1.472	1.484	1.523
Betaine 20-Shade	1.862	2.078	1.968	1.225	1.723	1.523	1.687	1.765	1.734
Betaine 40-Nil Shade	1.486	1.792	1.637	1.372	1.550	1.079	1.464	1.415	1.516
Betaine 40-Shade	1.633	1.854	1.742	1.309	1.599	1.518	1.584	1.709	1.627
Glycerol-Nil Shade	1.379	1.765	1.568	1.452	1.530	1.227	1.474	1.296	1.472
Glycerol-Shade	1.598	1.983	1.788	1.218	1.600	1.122	1.514	1.615	1.604
SE	0.165	0.125	0.082	0.107	0.053	0.136	0.057	0.106	0.044

[A] I – 30d = ADG from induction (I) to day 30; 30d – 60d = ADG from day 30 to day 60, and so on.
Means within a column within an effect with different superscripts are significantly different ($P < 0.05$). SE = standard error, d = days

4.9 Feed conversion efficiency

There was no dietary effect (P<0.05) on feed conversion ratio (dry matter feed intake:average daily gain) throughout the study period (Table 16). Over the entire study period (I – E), the Glycerol treatment recorded a numerically higher feed conversion ratio than the other dietary treatments. There was a shade effect for days I – 60 (P<0.05), 90 – 120 (P<0.01) and overall (I – E, P<0.01) where shaded treatments recorded lower fed conversion ratios. There were no diet × shade interactions. Overall feed conversion ratios matched those of commercial practice.

Table 16 Effect of diet (Betaine 10 (10 g/steer/d), 20 (20 g/steer/d) or 40 (40 g/steer/d)) or glycerol (5% DMB) and shade (no shade and shade) and their interaction on feed conversion ratio (dry matter feed intake: average daily gain)

	Feed conversion ratio						
	I-30d[A]	30d-60d	I-60d	60d-90d	I-90d	90d-120d	I-E
Diet (D)							
Control	6.63	5.60	5.99	7.37	6.39	6.60	6.43
Betaine 10	5.57	5.94	5.66	7.79	6.24	6.68	6.35
Betaine 20	5.53	5.64	5.55	8.37	6.26	6.56	6.32
Betaine 40	5.78	5.74	5.74	7.86	6.31	6.71	6.40
Glycerol	6.18	5.56	5.85	8.02	6.43	7.47	6.64
SE	0.47	0.24	0.17	0.50	0.16	0.28	0.11
Shade (S)							
Nil Shade	6.37	5.67	5.94[a]	7.51	6.41	7.29[a]	6.60[a]
Shade	5.51	5.72	5.57[b]	8.26	6.24	6.32[b]	6.25[b]
SE	0.30	0.15	0.11	0.32	0.10	0.18	0.07
D x S							
Control-Nil Shade	6.96	5.39	6.00	7.69	6.51	6.80	6.56
Control-Shade	6.30	5.81	5.98	7.06	6.26	6.41	6.30
Betaine 10-Nil Shade	6.47	5.57	5.89	7.37	6.34	7.20	6.54
Betaine 10-Shade	4.67	6.32	5.44	8.21	6.15	6.17	6.15
Betaine 20-Nil Shade	5.92	5.92	5.90	7.66	6.40	6.97	6.54
Betaine 20-Shade	5.15	5.35	5.20	9.09	6.12	6.15	6.11
Betaine 40-Nil Shade	6.10	5.73	5.89	7.57	6.37	7.17	6.56
Betaine 40-Shade	5.47	5.75	5.58	8.15	6.26	6.24	6.25
Glycerol-Nil Shade	6.40	5.75	6.04	7.26	6.43	8.32	6.83
Glycerol-Shade	5.97	5.38	5.65	8.78	6.43	6.62	6.45
SE	0.67	0.34	0.24	0.71	0.23	0.40	0.15

[A] I – 30d = feed conversion ratio from induction (I) to day 30; 30d – 60d = ADG from day 30 to day 60, and so on.
Means within a column within an effect with different superscripts are significantly different (*P < 0.05*). SE = standard error, d = days

There was no dietary effect (P<0.05) on inverse feed conversion ratio (average daily gain:dry matter intake) throughout the study period (Table 17). Over the entire study period (I-E), the Glycerol treatment recorded a numerically lower inverse feed conversion ratio than the other dietary treatments. There was a shade effect for days I – 30 (P<0.05), I – 60 (P<0.05), 90 – 120 (P<0.01) and overall (I – E, P<0.01)) where shaded treatments recorded higher inverse feed conversion ratios. There were no diet × shade interactions.

Table 17 Effect of diet (Betaine 10 (10 g/steer/d), 20 (20 g/steer/d) or 40 (40 g/steer/d)) or glycerol (5% DMB) and shade (no shade and shade) and their interaction on the inverse feed conversion ratio (average daily gain: dry matter intake)

	Inverse feed conversion ratio						
	I-30d[A]	30d-60d	I-60d	60d-90d	I-90d	90d-120d	I-E
Diet (D)							
Control	0.155	0.179	0.168	0.137	0.157	0.152	0.156
Betaine 10	0.187	0.170	0.177	0.129	0.160	0.151	0.158
Betaine 20	0.185	0.178	0.181	0.122	0.160	0.153	0.158
Betaine 40	0.174	0.175	0.175	0.129	0.158	0.150	0.156
Glycerol	0.162	0.180	0.171	0.127	0.156	0.137	0.151
SE	0.013	0.007	0.005	0.008	0.004	0.006	0.003
Shade (S)							
Nil Shade	0.159[b]	0.177	0.168[b]	0.134	0.156	0.138[b]	0.152[b]
Shade	0.186[a]	0.176	0.180[a]	0.123	0.160	0.159[a]	0.160[a]
SE	0.008	0.005	0.003	0.005	0.003	0.004	0.002
D x S							
Control-Nil Shade	0.147	0.185	0.167	0.131	0.154	0.148	0.153
Control-Shade	0.163	0.172	0.168	0.144	0.160	0.156	0.159
Betaine 10-Nil Shade	0.158	0.181	0.170	0.136	0.158	0.140	0.153
Betaine 10-Shade	0.215	0.160	0.185	0.122	0.163	0.162	0.163
Betaine 20-Nil Shade	0.170	0.169	0.170	0.133	0.157	0.144	0.153
Betaine 20-Shade	0.199	0.188	0.193	0.111	0.164	0.163	0.164
Betaine 40-Nil Shade	0.164	0.175	0.170	0.133	0.157	0.140	0.153
Betaine 40-Shade	0.184	0.176	0.179	0.124	0.160	0.160	0.160
Glycerol-Nil Shade	0.156	0.174	0.166	0.138	0.156	0.121	0.146
Glycerol-Shade	0.168	0.186	0.177	0.115	0.156	0.153	0.155
SE	0.019	0.010	0.007	0.012	0.006	0.009	0.004

[A] I – 30d = inverse feed conversion ratio from induction (I) to day 30; 30d – 60d = ADG from day 30 to day 60, and so on. Means within a column within an effect with different superscripts are significantly different ($P < 0.05$). SE = standard error, d = days

4.10 Visual hip height and change in visual hip height

Dietary treatment had no effect (P>0.05) on visual hip height throughout the study period (Table 18). The provision of shade resulted in an increase (P<0.05) in visual hip height for the overall (I – E) study period. There were no diet × shade interactions.

Table 18 Effect of diet (Betaine 10 (10 g/steer/d), 20 (20 g/steer/d) or 40 (40 g/steer/d)) or glycerol (5% DMB) and shade (no shade and shade) and their interaction on visual steer hip height from induction to exit (120 days)

	Visual hip height (mm)					
	I	30d	60d	90d	110d	E
Diet (D)						
Control	1259	1274	1294	1330	1344	1349
Betaine 10	1263	1280	1307	1335	1347	1355
Betaine 20	1271	1279	1300	1333	1352	1357
Betaine 40	1255	1277	1300	1330	1347	1349
Glycerol	1269	1274	1313	1333	1348	1350
SE	4	4	5	4	4	4
Shade (S)						
Nil Shade	1264	1277	1299	1329	1346	1348[b]
Shade	1262	1277	1306	1336	1349	1356[a]
SE	3	3	3	3	2	3
D x S						
Control-Nil Shade	1263	1270	1288	1324	1340	1338
Control-Shade	1256	1277	1299	1337	1349	1361
Betaine 10-Nil Shade	1265	1280	1304	1327	1343	1351
Betaine 10-Shade	1260	1280	1311	1342	1352	1359
Betaine 20-Nil Shade	1263	1280	1299	1335	1355	1356
Betaine 20-Shade	1279	1278	1302	1332	1349	1357
Betaine 40-Nil Shade	1254	1282	1299	1332	1350	1350
Betaine 40-Shade	1256	1273	1301	1329	1343	1348
Glycerol-Nil Shade	1276	1271	1307	1327	1341	1345
Glycerol-Shade	1262	1276	1319	1339	1355	1356
SE	6	6	7	6	5	6

Means within a column within an effect with different superscripts are significantly different (*P < 0.05*).
SE = standard error, d = days

Dietary treatment resulted in an increased change in visual hip height (P<0.05) of the Glycerol treatment compared to other treatments from days 30 – 60 (Table 19). However, from days 60 – 90, the Glycerol treatment recorded a decreased change in visual hip height (P<0.05) compared to all other treatments except Betaine 10. Note that based on the study design, the glycerol effect was due to either glycerol *per se* or the pen position. The provision of shade resulted in an increase (P<0.05) in change in visual hip height for the overall (I – E) study period. There were no diet × shade interactions. On average, the steers gained 89 mm in visual hip height over the study period.

Table 19 Effect of diet (Betaine 10 (10 g/steer/d), 20 (20 g/steer/d) or 40 (40 g/steer/d)) or glycerol (5% DMB) and shade (no shade and shade) and their interaction on the change in visual steer hip height from induction to exit (120 days)

	Change in hip height (mm)								
	I-30d	30d-60d	I-60d	60d-90d	I-90d	90d-110d	I-110d	90d-E	I-E
Diet (D)									
Control	11.2[A]	19.7[b]	31.0	36.6[a]	67.5	14.1	81.7	19.1	86.7
Betaine 10	17.6	27.2[b]	44.8	27.1[ab]	71.9	12.7	84.6	20.2	92.1
Betaine 20	16.0	21.7[b]	37.7	33.0[a]	70.8	18.6	89.4	23.4	94.1
Betaine 40	14.6	22.5[b]	37.2	30.5[a]	67.6	16.3	83.9	18.6	86.3
Glycerol	11.0	39.3[a]	50.3	20.3[b]	70.7	14.3	85.0	17.0	87.7
SE	4.5	3.7	4.7	3.2	4.0	3.2	3.9	2.8	4.0
Shade (S)									
Nil Shade	14.0	22.8	36.8	29.5	66.3	16.8	83.1	19.0	85.2[b]
Shade	14.2	29.4	43.6	29.6	73.2	13.6	86.7	20.4	93.5[a]
SE	2.8	2.4	3.0	2.1	2.5	2.0	2.5	1.8	2.5
D x S									
Control-Nil Shade	7.6	17.8	25.4	35.7	61.1	15.9	77.0	14.0	75.1
Control-Shade	14.8	21.7	36.6	37.5	74.0	12.4	86.4	24.2	98.2
Betaine 10-Nil Shade	17.4	23.9	41.3	23.1	64.4	16.0	80.4	23.5	88.0
Betaine 10-Shade	17.7	30.6	48.3	31.1	79.4	9.5	88.9	16.8	96.2
Betaine 20-Nil Shade	17.0	19.3	36.3	35.7	72.0	20.6	92.6	21.8	93.8
Betaine 20-Shade	15.1	24.1	39.0	30.4	69.6	16.6	86.1	24.9	94.4
Betaine 40-Nil Shade	19.1	17.1	36.3	32.9	69.1	18.6	87.7	17.8	86.9
Betaine 40-Shade	10.1	27.9	38.1	28.2	66.2	14.0	80.2	19.5	85.7
Glycerol-Nil Shade	8.8	35.9	44.5	20.1	64.7	13.2	77.9	17.7	82.4
Glycerol-Shade	13.2	42.8	56.0	20.6	76.6	15.4	92.0	16.3	92.9
SE	6.3	5.2	6.6	4.5	5.6	4.5	5.4	3.9	5.6

[A] I – 30d =change in hip height from induction (I) to day 30; 30d – 60d = ADG from day 30 to day 60, and so on.
Means within a column within an effect with different superscripts are significantly different ($P < 0.05$). SE = standard error, d = days

4.11 US body condition score

Dietary treatment influenced US Body Condition Score (USBCS) with the Control treatment having a lower USBCS (P<0.05) than Betaine 40 and the Glycerol treatment having a lower USBCS (P<0.05) than Betaine 20 and 40 at 90 days (Table 20). Note that based on the study design, the glycerol effect was due to either glycerol *per se* or the pen position. At day 60, the provision of shade resulted in an increased USBCS (P<0.05). There were no diet × shade interactions. At an average score of 7.35 at Exit, the steers were in a marketable body condition.

Table 20 Effect of diet (Betaine 10 (10 g/steer/d), 20 (20 g/steer/d) or 40 (40 g/steer/d)) or glycerol (5% DMB) and shade (no shade and shade) and their interaction on steer body condition score (USBCS) from induction (I) to exit (E) at 120 days on feed

	USBCS					
	I	30d	60d	90d	110d	E
Diet (D)						
Control	5.2	5.5	6.0	6.8^{bc}	7.0	7.3
Betaine 10	5.3	5.6	6.2	7.0^{ab}	7.2	7.3
Betaine 20	5.2	5.6	6.2	7.1^{ac}	7.2	7.3
Betaine 40	5.3	5.7	6.3	7.2^{a}	7.3	7.5
Glycerol	5.2	5.5	6.0	6.8^{b}	7.0	7.2
SE	0.1	0.1	0.1	0.1	0.1	0.1
Shade (S)						
Nil Shade	5.2	5.5	6.0^{b}	7.0	7.1	7.3
Shade	5.2	5.6	6.3^{a}	7.0	7.2	7.4
SE	0.0	0.0	0.1	0.0	0.1	0.1
D x S						
Control-Nil Shade	5.2	5.4	5.9	6.9	7.0	7.2
Control-Shade	5.2	5.6	6.1	6.8	7.1	7.4
Betaine 10-Nil Shade	5.2	5.7	6.0	7.0	7.2	7.3
Betaine 10-Shade	5.3	5.6	6.4	7.0	7.3	7.3
Betaine 20-Nil Shade	5.2	5.6	6.1	7.0	7.0	7.3
Betaine 20-Shade	5.1	5.7	6.3	7.1	7.4	7.4
Betaine 40-Nil Shade	5.3	5.6	6.2	7.0	7.2	7.3
Betaine 40-Shade	5.2	5.8	6.5	7.3	7.4	7.6
Glycerol-Nil Shade	5.1	5.4	5.9	6.8	7.0	7.2
Glycerol-Shade	5.4	5.5	6.1	6.8	7.0	7.1
SE	0.1	0.1	0.1	0.1	0.1	0.1

Means within a column within an effect with different superscripts are significantly different (*P < 0.05*). SE = standard error, d = days

There was no dietary effect on the change (P>0.05) in the change in USBCS during the study period (Table 21). The Glycerol treatment recorded the numerically lowest change in USBCS over the I – E period. The provision of shade resulted in an increased change (P<0.05) in USBCS between days 30 – 60, I – 60 and days 60 – 90. There were no diet × shade interactions. On average, the steers increased their body condition by two scores over the study period.

Table 21 Effect of diet (Betaine 10 (10 g/steer/d), 20 (20 g/steer/d) or 40 (40 g/steer/d)) or glycerol (5% DMB) and shade (no shade and shade) and their interaction on change in steer US body condition score (USBCS) from induction (I) to exit (E) at 120 days on feed

	Change in USBCS								
	I-30d	30d-60d	I-60d	60d-90d	I-90d	90d-110d	I-110d	90d-E	I-E
Diet (D)									
Control	0.3[A]	0.5	0.8	0.8	1.6	0.2	1.9	0.5	2.1
Betaine 10	0.4	0.6	1.0	0.8	1.7	0.2	1.9	0.3	2.0
Betaine 20	0.4	0.6	1.1	0.9	1.9	0.1	2.0	0.3	2.2
Betaine 40	0.5	0.6	1.1	0.8	1.9	0.1	2.0	0.3	2.2
Glycerol	0.2	0.6	0.8	0.8	1.6	0.2	1.8	0.3	1.9
SE	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Shade (S)									
Nil Shade	0.3	0.5[b]	0.8[b]	0.9[a]	1.7	0.1	1.9	0.3	2.1
Shade	0.4	0.7[a]	1.0[a]	0.7[b]	1.8	0.2	2.0	0.4	2.1
SE	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1
D x S									
Control-Nil Shade	0.2	0.5	0.7	1.0	1.7	0.1	1.8	0.4	2.0
Control-Shade	0.4	0.5	0.9	0.7	1.6	0.3	1.9	0.6	2.2
Betaine 10-Nil Shade	0.4	0.4	0.8	1.0	1.8	0.1	1.9	0.3	2.1
Betaine 10-Shade	0.3	0.8	1.1	0.6	1.7	0.3	2.0	0.3	2.0
Betaine 20-Nil Shade	0.4	0.5	0.9	0.9	1.8	0.0	1.8	0.3	2.1
Betaine 20-Shade	0.5	0.7	1.2	0.8	2.0	0.2	2.2	0.3	2.3
Betaine 40-Nil Shade	0.3	0.6	0.9	0.8	1.7	0.2	1.9	0.3	2.0
Betaine 40-Shade	0.6	0.6	1.2	0.8	2.0	0.1	2.1	0.3	2.4
Glycerol-Nil Shade	0.3	0.5	0.8	0.9	1.7	0.1	1.8	0.4	2.1
Glycerol-Shade	0.1	0.7	0.8	0.7	1.5	0.2	1.7	0.3	1.8
SE	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1

[A] I – 30d =change in US body condition score from induction (I) to day 30; 30d – 60d = ADG from day 30 to day 60, and so on.
Means within a column within an effect with different superscripts are significantly different ($P < 0.05$). SE = standard error, d = days

4.12 Body temperature

The glycerol fed steers had a higher (P<0.05) body temperature than the other dietary treatments between days 1 and 30 (Table 22). Apart from days 1 – 30 there were no other differences in body temperature of steers with implanted temperature transmitters due to diet. Throughout the study period, the Glycerol treatment recorded a numerically higher body temperature than the other treatments. The provision of shade had no effect (P>0.05) on body temperature. There were no diet × shade interactions.

Table 22 Effect of diet (Betaine 10 (10 g/steer/d), 20 (20 g/steer/d) or 40 (40 g/steer/d)) or glycerol (5% DMB) and shade (no shade and shade) and their interaction on the body temperature of steers implanted with temperature transmitters (° C) from induction (I) to 120 days on feed

	Body temperature (°C)			
	I-30d[A]	30d-60d	60d-90d	90d-120d
Diet (D)				
Control	39.56[b]	39.64	39.70	39.58
Betaine 10	39.49[b]	39.61	39.69	39.59
Betaine 20	39.53[b]	39.52	39.57	39.48
Betaine 40	39.39[b]	39.59	39.72	39.60
Glycerol	39.82[a]	39.84	39.81	39.69
SE	0.07	0.09	0.08	0.07
Shade (S)				
Nil Shade	39.54	39.60	39.75	39.59
Shade	39.57	39.68	39.65	39.58
SE	0.05	0.06	0.05	0.05
D x S				
Control-Nil Shade	39.53	39.61	39.74	39.53
Control-Shade	39.59	39.68	39.67	39.64
Betaine 10-Nil Shade	39.48	39.53	39.69	39.56
Betaine 10-Shade	39.50	39.69	39.69	39.62
Betaine 20-Nil Shade	39.55	39.42	39.59	39.48
Betaine 20-Shade	39.51	39.62	39.55	39.49
Betaine 40-Nil Shade	39.32	39.49	39.75	39.61
Betaine 40-Shade	39.46	39.69	39.70	39.59
Glycerol-Nil Shade	39.84	39.96	39.96	39.80
Glycerol-Shade	39.79	39.72	39.65	39.58
SE	0.10	0.13	0.11	0.10

[A] I – 30d =steer body temperature from induction (I) to day 30; 30d – 60d = ADG from day 30 to day 60, and so on.
Means within a column within an effect with different superscripts are significantly different (*P < 0.05*). SE = standard error, d = days

The magnitude of body temperature values in steers implanted with temperature transmitters increased across time with numerically higher means reported between days 60 and 90. There was a marked diurnal variation in some individual steer body temperatures of up to 5 °C recorded. This occurred over a range of 37 °C to 42.8 °C, with the upper values recorded during 'hot' periods. This range and upper value is in agreement with Gaughan *et al*. (1999; 2008b) and is much greater than reported by Loxton *et al.* (2007) during similar months at the same location in 2006. The normal range in temperature is 38.6 – 39.0 °C at thermoneutral conditions.

The relationship between the body temperature measured by the digital temperature transmitters used in the study and the standard rectal temperature was studied on three occasions during the study period. The temperature transmitters recorded higher temperatures with the mean difference being 0.38 ± 0.0255 ^{o}C (SE diff.), r=0.8726; 0.38 ± 0.0199 ^{o}C (SE diff.), r=0.9031 and 0.43 ± 0.0251 ^{o}C (SE diff.), r=0.9229 in December 2008, January and March 2009 respectively. The data suggests the difference between rectal temperature and body temperature measured by the digital temperature transmitters was consistent and repeatable.

From induction until January 10, 2008 (Day 59) a RFI 2.5 db gain end fed mobile dipole aerial mounted at 3 metres above ground level was used to receive radio pulses from the implanted digital temperature transmitters. During this period, digital temperature pulses were often missed from implanted steers in the farthest 4 treatment pens of the feedlot because of their distance from the receiver aerial and the animal's orientation to the aerial. To overcome this problem, a RFI 4.5 db gain base station Omni vertical collinear aerial was installed on January 10, 2008 at 10 m above ground level to receive the digital temperature pulses and replace the original RFI 2.5 db gain end fed mobile dipole aerial. This enhancement of aerials significantly increased the number of digital temperature pulse acquisitions in the farthest feedlot pens for the remainder of the study.

There was no dietary effect ($P>0.05$) on the range of steer body temperature throughout the study period (Table 23). The provision of shade reduced ($P<0.01$) the range in steer body temperature for I – 30d ($P<0.001$), 30d – 60d ($P<0.01$), 60d – 90d ($P<0.001$) and 90d – 120d ($P<0.001$). The range in steer body temperature was reduced by 26 to 37% due to the provision of shade. There were no diet × shade interactions.

Table 23 Effect of diet (Betaine 10 (10 g/steer/d), 20 (20 g/steer/d) or 40 (40 g/steer/d)) or glycerol (5% DMB) and shade (no shade and shade) and their interaction on the range of body temperature (° C) in steers with implanted temperature transmitters from induction (I) to 120 days on feed

	Range in body temperature (°C)			
	I-30d	30d-60d	60d-90d	90d-120d
Diet (D)				
Control	1.40[A]	1.43	1.50	1.33
Betaine 10	1.34	1.44	1.45	1.37
Betaine 20	1.59	1.64	1.60	1.40
Betaine 40	1.62	1.52	1.61	1.49
Glycerol	1.70	1.73	1.62	1.53
SE	0.10	0.15	0.07	0.09
Shade (S)				
Nil Shade	1.77[a]	1.78[a]	1.91[a]	1.65[a]
Shade	1.29[b]	1.32[b]	1.20[b]	1.20[b]
Pooled SE	0.06	0.09	0.05	0.06
D x S				
Control-Nil Shade	1.62	1.65	1.82	1.52
Control-Shade	1.17	1.21	1.18	1.14
Betaine 10-Nil Shade	1.51	1.70	1.83	1.61
Betaine 10-Shade	1.17	1.18	1.07	1.13
Betaine 20-Nil Shade	1.84	1.82	1.91	1.58
Betaine 20-Shade	1.33	1.46	1.29	1.22
Betaine 40-Nil Shade	1.74	1.54	1.88	1.65
Betaine 40-Shade	1.50	1.50	1.34	1.34
Glycerol-Nil Shade	2.12	2.21	2.13	1.87
Glycerol-Shade	1.28	1.26	1.10	1.18
SE	0.14	0.21	0.10	0.13

[A] I – 30d = range in steer body temperature data from induction (I) to day 30; 30d – 60d = ADG from day 30 to day 60, and so on. Means within a column within an effect with different superscripts are significantly different ($P < 0.05$). SE = standard error, d = days

4.13 Panting score

The change in panting scores i.e. from 0 to 4.5 as the animal is heat challenged is a good indicator of the changing heat load status of the animal (Mader *et al.,* 2006). When a group is assessed the mean panting score (MPS) is used (Brown-Brandl *et al.*, 2006; Gaughan *et al.*, 2008a). The MPS can be used as an indicator of the severity of climatic induced stress: 0 to 0.4 minimal heat load – no stress; 0.4 to 0.8 moderate heat load – slight stress; 0.8 to 1.2 high heat load – moderate heat load; >1.2 extreme heat load cattle highly stressed (Gaughan *et al.,* 2008c). It is clear from the data presented here that the cattle, especially un-shaded cattle were under extreme heat load at Midday.

The panting score of steers was not affected by diet (P>0.05) from Induction to day 30 (Table 24). The provision of shade reduced the panting score of steers in the morning (P<0.001), at Midday (P<0.001) and in the afternoon (P<0.001) between Induction and day 30. There were no diet × shade interactions. The highest magnitude of panting scores was recorded at the Midday observation.

Table 24 Effect of diet (Betaine 10 (10 g/steer/d), 20 (20 g/steer/d) or 40 (40 g/steer/d)) or glycerol (5% DMB) and shade (no shade and shade) and their interaction on steer panting score at three times of the day from Induction to Day 30

	Mean panting score		
	AM	Midday	PM
Diet (D)			
Control	0.78	1.40	0.91
Betaine 10	0.74	1.30	0.84
Betaine 20	0.74	1.35	0.87
Betaine 40	0.75	1.35	0.88
Glycerol	0.72	1.40	0.98
SE	0.03	0.04	0.04
Shade (S)			
Nil Shade	0.82[a]	1.57[a]	0.96[a]
Shade	0.67[b]	1.15[b]	0.83[b]
SE	0.02	0.03	0.03
D x S			
Control-Nil Shade	0.86	1.57	0.95
Control-Shade	0.71	1.23	0.88
Betaine 10-Nil Shade	0.84	1.55	0.93
Betaine 10-Shade	0.65	1.06	0.75
Betaine 20-Nil Shade	0.81	1.55	0.94
Betaine 20-Shade	0.66	1.14	0.80
Betaine 40-Nil Shade	0.80	1.55	0.94
Betaine 40-Shade	0.71	1.16	0.82
Glycerol-Nil Shade	0.81	1.64	1.05
Glycerol-Shade	0.63	1.16	0.91
SE	0.04	0.06	0.06

Means within a column within an effect with different superscripts are significantly different ($P < 0.05$). SE = standard error

Dietary treatment influenced the panting score of steers at both the morning and Midday observations between days 30 and 60 (Table 25). The Glycerol treatment had a lower morning panting score (P<0.05) than Control, Betaine 10 and Betaine 40, while Betaine 20 had a lower panting score than Control and Betaine 40 (P<0.05). Note that based on the study design, the glycerol effect was due to either glycerol *per se* or the pen position. The Control treatment had a higher Midday panting score (P<0.05) than all other treatments. The provision of shade lowered the panting score in the morning (P<0.05), at Midday (P<0.001) and in the afternoon (P<0.001) between days 30 and 60. There were no diet × shade interactions. The highest magnitude of panting scores was recorded at the Midday observation.

Table 25 Effect of diet (Betaine 10 (10 g/steer/d), 20 (20 g/steer/d) or 40 (40 g/steer/d)) or glycerol (5% DMB) and shade (no shade and shade) and their interaction on steer panting score at three times of the day from Day 30 to Day 60

	Mean panting score		
	AM	Midday	PM
Diet (D)			
Control	0.64[a]	1.52[a]	0.88
Betaine 10	0.61[ab]	1.40[b]	0.84
Betaine 20	0.51[bc]	1.34[b]	0.75
Betaine 40	0.62[a]	1.39[b]	0.83
Glycerol	0.50[c]	1.39[b]	0.81
SE	0.03	0.04	0.04
Shade (S)			
Nil Shade	0.61[a]	1.61[a]	0.89[a]
Shade	0.54[b]	1.21[b]	0.75[b]
SE	0.02	0.03	0.03
D x S			
Control-Nil Shade	0.66	1.66	0.89
Control-Shade	0.62	1.39	0.87
Betaine 10-Nil Shade	0.66	1.63	0.95
Betaine 10-Shade	0.56	1.16	0.73
Betaine 20-Nil Shade	0.56	1.53	0.82
Betaine 20-Shade	0.47	1.15	0.69
Betaine 40-Nil Shade	0.66	1.61	0.89
Betaine 40-Shade	0.57	1.18	0.77
Glycerol-Nil Shade	0.53	1.63	0.93
Glycerol-Shade	0.47	1.15	0.69
SE	0.05	0.05	0.05

Means within a column within an effect with different superscripts are significantly different (*P < 0.05*). SE = standard error

The Glycerol treatment had a lower AM panting score (P<0.01) than all other treatments between days 60 to 90 (Table 26). Note that based on the study design, the glycerol effect was due to either glycerol per se or the pen position. Dietary treatment had no influence (P>0.05) on Midday or PM panting scores over days 60 to 90. The provision of shade lowered the panting score (P<0.001) at each observation time between days 60 and 90. There were no diet × shade interactions. The highest magnitude of panting scores was recorded at the Midday observation.

Table 26 Effect of diet (Betaine 10 (10 g/steer/d), 20 (20 g/steer/d) or 40 (40 g/steer/d)) or glycerol (5% DMB) and shade (no shade and shade) and their interaction on steer panting score at three times of the day from Day 60 to Day 90

	Mean panting score		
	AM	Midday	PM
Diet (D)			
Control	0.57[a]	1.37	0.72
Betaine 10	0.55[a]	1.24	0.70
Betaine 20	0.53[a]	1.22	0.63
Betaine 40	0.57[a]	1.29	0.69
Glycerol	0.42[b]	1.27	0.72
SE	0.02	0.04	0.02
Shade (S)			
Nil Shade	0.60[a]	1.52[a]	0.77[a]
Shade	0.45[b]	1.04[b]	0.61[b]
SE	0.02	0.03	0.02
D x S			
Control-Nil Shade	0.63	1.59	0.79[1]
Control-Shade	0.50	1.15	0.66
Betaine 10-Nil Shade	0.64	1.51	0.78
Betaine 10-Shade	0.46	0.97	0.61
Betaine 20-Nil Shade	0.60	1.43	0.68
Betaine 20-Shade	0.45	1.02	0.58
Betaine 40-Nil Shade	0.62	1.51	0.76
Betaine 40-Shade	0.51	1.06	0.63
Glycerol-Nil Shade	0.49	1.55	0.87
Glycerol-Shade	0.35	1.00	0.57
SE	0.03	0.05	0.03

[1]Significant diet × shade interaction (P<0.05). Means within a column within an effect with different superscripts are significantly different (*P < 0.05*). SE = standard error

The Glycerol treatment had a lower morning panting score (P<0.01) than all other treatments between days 90 to 120 (Table 27). Note that based on the study design, the glycerol effect was due to either glycerol *per se* or the pen position. The Control treatment had a higher Midday panting score (P<0.05) than all treatments except Betaine 20. The provision of shade lowered the panting score (P<0.001) at each observation time between days 90 and 120. There was no diet × shade interaction for AM and Midday scores, however the diet × shade interaction for afternoon scores was significant (P<0.05). The highest magnitude of panting scores was recorded at the Midday observation.

Table 27 Effect of diet (Betaine 10 (10 g/steer/d), 20 (20 g/steer/d) or 40 (40 g/steer/d)) or glycerol (5% DMB) and shade (no shade and shade) and their interaction on steer panting score at three times of the day from Day 90 to Day 120

	Mean panting score		
	AM	Midday	PM
Diet (D)			
Control	0.31[a]	1.32[a]	0.72
Betaine 10	0.29[a]	1.20[b]	0.68
Betaine 20	0.27[a]	1.16[b]	0.63
Betaine 40	0.30[a]	1.24[ab]	0.69
Glycerol	0.17[b]	1.21[b]	0.67
SE	0.02	0.03	0.03
Shade (S)			
Nil Shade	0.32[a]	1.42[a]	0.74[a]
Shade	0.22[b]	1.03[b]	0.62[b]
Pooled SE	0.01	0.02	0.02
D x S			
Control-Nil Shade	0.37	1.48	0.74
Control-Shade	0.25	1.17	0.70
Betaine 10-Nil Shade	0.35	1.40	0.74
Betaine 10-Shade	0.23	0.99	0.62
Betaine 20-Nil Shade	0.31	1.34	0.66
Betaine 20-Shade	0.22	0.98	0.60
Betaine 40-Nil Shade	0.34	1.43	0.73
Betaine 40-Shade	0.26	1.05	0.66
Glycerol-Nil Shade	0.21	1.46	0.82
Glycerol-Shade	0.13	0.97	0.52
SE	0.03	0.04	0.04

Means within a column within an effect with different superscripts are significantly different ($P < 0.05$). SE = standard error

The diet × shade interaction (P<0.05) between days 90 and 120 for afternoon panting score is shown in Figure 5. The interaction is due to the divergence in panting score as a consequence of the influence of shade for the Glycerol treatment compared to the other treatments.

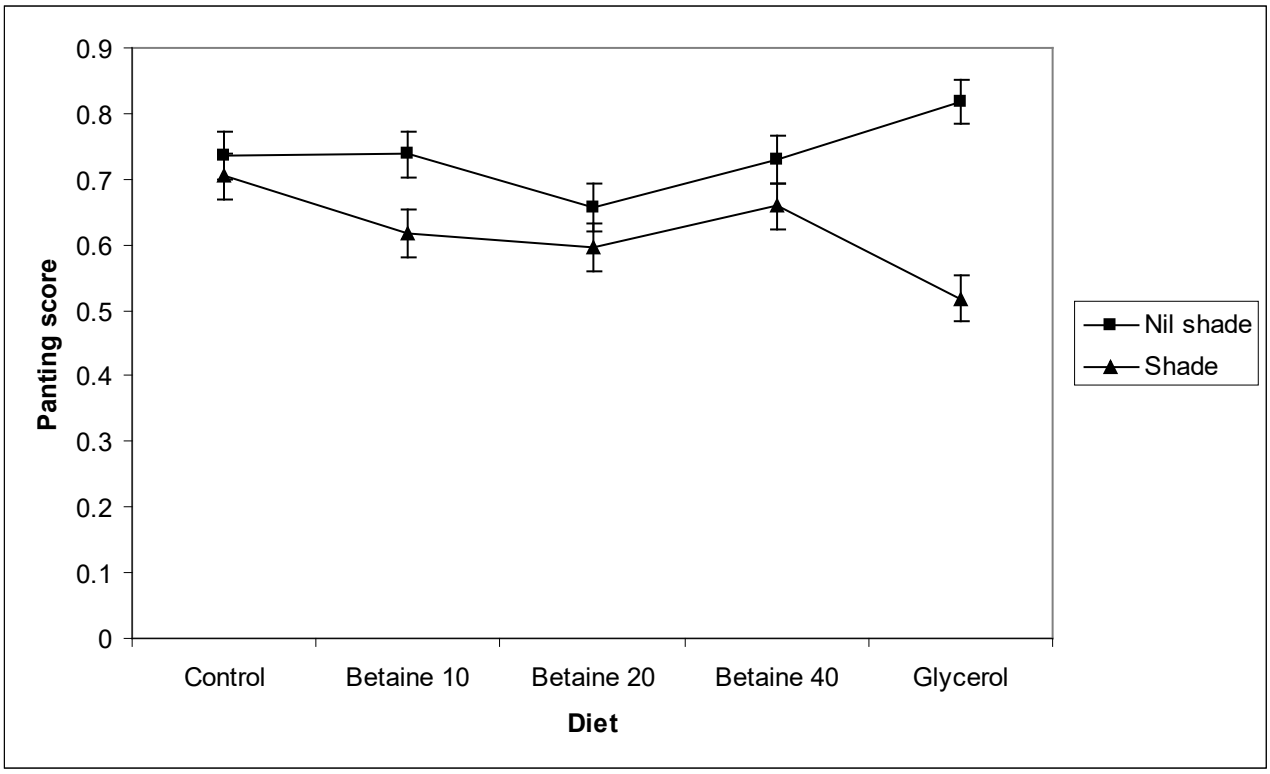

Figure 5 Diet × shade interaction of PM panting score for days 90 to 120

Panting scores were always lowest in the morning, intermediate in the afternoon and highest at Midday throughout the study period. Although the lowest scores were observed in the morning, the differences due to dietary treatment were generally recorded in the morning – notably generally due to the Glycerol treatment. The provision of shade reduced panting scores at each observation time across the entire study period.

The lower morning panting score of the Glycerol treatment over most of the study period is of interest, as it is in contrast to the numerically higher body temperatures recorded for this treatment. This result suggests the Glycerol treatment steers had a better coping physiological coping mechanism as they were able to shed heat overnight resulting in lower AM panting scores. This is supported somewhat as the Glycerol treatment recorded a higher numerical body temperature range over the study period.

4.14 Blood metabolite, chemistry and haematology

There were no significant diet or diet × month effects ($P>0.05$) for either heat shock protein 70 (Hsp70) or the haematology parameters (Table 28), however month effects were significant ($P<0.05$) for all parameters except basophils. For plasma biochemistry (Table 29), there were no significant diet effects ($P>0.05$), however similar to the haematology data, month effects were significant ($P<0.05$) for all parameters except chloride (Cl). Diet × month interactions were significant ($P<0.05$) for parameters including creatinine, sodium (Na) and Cl. Plasma creatinine concentrations were significantly different between dietary treatments on 30, 60 and 90 days on feed (DOF), corresponding to December, January and February respectively. For days 30, 60 and 90, plasma creatinine concentration was lower ($P<0.05$) for betaine 10 treatment, and was higher ($P<0.05$) for betaine 40 treatment (December only). Betaine 10 treatment displayed numerically lower ($P>0.05$) creatinine concentration on days 0 and 110,

supporting the trend observed for days 30, 60 and 90. Furthermore, creatinine concentration was numerically higher ($P>0.05$) for betaine 40 treatment on days 60, 90 and 110, in addition to higher ($P<0.05$) creatinine measured for betaine 40 on day 30.

Month effects were significant ($P<0.05$) for both Hsp70 and haematology parameters (Table 30) for analysis of shade within month, however the shade and shade × month effects were not significant ($P>0.05$). Similarly for plasma biochemistry parameters (Table 31) month effects were significant ($P<0.05$) for analysis of shade within month. Also for plasma biochemistry, shade and shade × month effects were non-significant ($P>0.05$), except for Na and Cl. For Na, differences ($P<0.05$) between shaded and non-shaded treatments were observed on days 30, 60 and 90 (December, January and February respectively), while for Cl, differences ($P<0.05$) were observed on day 30.

Table 28 Effect of diet (Betaine 10 (10 g/steer/d), 20 (20 g/steer/d) or 40 (40 g/steer/d)) or glycerol (5% DMB) within month on plasma concentration of heat shock protein 70 (hsp70; optical density OD) and blood haematological parameters, sampled from steers with temperature transmitters at 0 (induction), 30, 60, 90 or 110 days on feed (DOF)

Month [DOF]	Diet	Hsp70 (OD)	WBC[A] (x10e9/L)	Neutrophil (N) (%)	Lymphocyte (L) (%)	N:L (%)	Monocyte (%)	Eosinophil (%)	Basophil (%)	RBC[B] (x10e12/L)	HGB[C] (g/L)	HCT[D] (%)	Platelets (x10e9/L)
November	Control	0.31	11.7	27.8	62.8	0.58	6.79	0.74	1.93	7.52	113.6	26.5	165.6
[0]	Betaine 10	0.14	11.3	26.9	61.8	0.47	8.48	0.80	2.00	7.23	105.0	25.9	167.5
	Betaine 20	0.17	10.6	31.2	59.8	0.66	6.09	0.80	2.11	7.34	110.7	25.8	137.8
	Betaine 40	0.16	10.4	33.7	56.3	0.69	7.22	1.33	1.48	6.8	109.3	25.4	222.3
	Glycerol	0.16	12.3	20.9	71.8	0.32	5.31	0.57	1.39	7.13	110.5	25.5	143.4
	SE	0.19	1.0	3.5	4.3	0.11	1.08	0.51	4.06	0.37	5.8	1.2	52.8
December	Control	1.13	11.2	27.7	61.7	0.62	7.70	2.10	1.01	6.42	116.0	27.1	220.8
[30]	Betaine 10	0.67	12.3	22.5	69.3	0.38	5.57	0.99	1.11	6.48	118.0	26.9	377.7
	Betaine 20	0.82	11.3	22.5	67.9	0.38	7.20	1.28	1.37	5.85	111.4	25.8	337.4
	Betaine 40	1.07	10.3	24.3	64.5	0.43	9.24	0.88	1.37	5.51	105.7	24.9	277.7
	Glycerol	0.94	9.6	23.1	68.5	0.32	6.36	1.16	1.20	6.36	111.9	26.2	407.3
	SE	0.25	1.3	4.8	5.9	0.16	1.59	0.74	5.98	0.47	7.0	1.4	75.7
January	Control	1.01	11.6	21.2	70.9	0.33	4.09	2.44	1.57	7.38	131.3	30.5	214.0
[60]	Betaine 10	0.38	11.5	19.9	72.0	0.31	4.47	2.41	0.98	7.18	131.2	30.0	302.0
	Betaine 20	0.94	15.5	13.5	81.2	0.08	3.44	1.20	1.31	6.62	122.7	28.9	124.4
	Betaine 40	0.78	11.4	18.6	73.5	0.14	5.19	1.67	1.84	6.54	126.8	29.2	75.4
	Glycerol	0.90	11.4	14.7	77.4	0.20	4.12	2.26	1.54	7.17	127.0	29.4	333.7
	SE	0.29	1.6	5.7	6.9	0.19	1.93	0.90	7.30	0.53	7.8	1.6	90.8
February	Control	0.80	9.4	34.1	51.9	0.77	7.27	4.41	2.56	8.24	137.8	31.5	301.5
[90]	Betaine 10	0.23	12.0	23.0	69.0	0.39	3.90	2.00	1.66	7.79	136.9	30.8	322.8
	Betaine 20	0.84	10.0	29.6	61.3	0.54	3.75	2.52	3.10	6.93	136.9	28.3	116.5
	Betaine 40	0.75	10.6	26.2	63.2	0.46	6.81	1.66	2.45	7.7	136.2	31.2	118.6
	Glycerol	0.38	10.4	20.1	64.4	0.31	11.26	2.96	34.50	7.47	129.9	29.2	323.0
	SE	0.26	1.3	4.8	5.9	0.16	1.59	0.74	5.98	0.47	7.0	1.4	75.7
March	Control	0.77	11.8	22.8	70.6	0.35	2.90	2.08	1.59	8.26	136.1	31.4	241.3
[110]	Betaine 10	0.19	10.6	24.6	68.2	0.40	3.14	2.58	1.47	8.21	139.6	31.9	201.7

	Betaine 20	0.59	9.8	30.4	62.4	0.54	4.05	1.26	2.93	8.33	142.3	32.6	254.7
	Betaine 40	0.48	7.4	38.1	51.9	0.92	6.63	2.73	2.17	7.99	137.0	31.6	299.7
	Glycerol	0.26	11.0	17.4	76.3	0.24	3.64	1.27	1.32	7.91	132.2	30.6	221.3
	SE	0.19	1.0	3.5	4.3	0.11	1.07	0.51	4.02	0.37	5.8	1.2	52.3
F-prob	Diet (D)	0.35	0.70	0.30	0.40	0.30	0.33	0.44	0.54	0.66	1.00	0.91	0.51
	Month (M)	<0.001	0.002	<0.001	<0.001	0.005	<0.001	<0.001	0.1607	<0.001	<0.001	<0.001	<0.001
	D × M	0.86	0.29	0.28	0.24	0.16	0.10	0.40	0.32	0.77	0.76	0.74	0.24

[A] White Blood Cells
[B] Red Blood Cells
[C] Haemoglobin
[D] Haematocrit
Standard error (SE) is presented as a pooled value for individual parameters (columns) within each month.

Table 29 Effect of diet (betaine 10 (10 g/steer/d), 20 (20 g/steer/d) or 40 (40 g/steer/d)) or glycerol (5% DMB) within month on plasma concentration of biochemistry parameters, sampled from steers with temperature transmitters at 0 (induction), 30, 60, 90 or 110 days on feed (DOF)

Month	Diet	Prot[A] g/L	Alb[B] ng/L	Glo[C] g/L	Alb:Glo[D] ratio	CPK[E] U/L	Glucose umol/L	LDH[F] U/L	Urea umol/L	Creat[G] umol/L	K mmol/L	Cl mmol/L	Na mmol/L
November	Control	72.6	36.2	36.4	1.01	146.3	4.95	914.9	3.50	159.2^{a}	4.60	105.3^{a}	133.4^{a}
[0 DOF)	Betaine 10	71.3	34.4	36.9	0.94	281.8	4.66	887.8	3.16	151.7^{a}	4.23	105.7^{a}	128.6^{b}
	Betaine 20	73.1	34.5	38.6	0.90	1773.7	4.66	920.7	3.57	160.7^{a}	4.49	106.4^{a}	131.1^{ab}
	Betaine 40	73.5	35.6	37.8	0.95	358.4	4.57	909.8	3.35	158.2^{a}	4.48	105.5^{a}	131.5^{ab}
	Glycerol	74.2	34.8	39.4	0.89	354.9	4.73	879.9	3.42	163.4^{a}	4.30	106.2^{a}	129.4^{b}
	SE	1.0	0.4	1.0	0.03	221.8	0.16	52.0	0.28	6.6	0.09	2.2	1.2
December	Control	72.3	35.4	36.9	0.97	219.1	4.95	826.5	6.37	117.5^{a}	4.41	90.2^{b}	133.0^{a}
[30]	Betaine 10	72.0	33.8	38.0	0.90	110.4	5.04	827.7	5.26	113.8^{ac}	4.36	109.3^{a}	132.5^{a}
	Betaine 20	73.8	33.3	40.5	0.83	187.1	4.84	796.1	5.33	142.8^{ad}	4.27	107.1^{a}	131.6^{a}
	Betaine 40	76.0	33.6	42.2	0.80	202.8	4.84	921.6	5.86	171.9^{b}	4.22	108.0^{a}	134.7^{a}
	Glycerol	73.9	34.8	39.0	0.91	210.4	5.18	925.0	5.34	130.3^{a}	4.37	114.5^{a}	126.9^{b}
	SE	1.3	0.6	1.3	0.04	321.0	0.19	72.3	0.39	8.9	0.13	3.3	1.2
January	Control	75.1	36.1	39.0	0.93	120.6	5.20	910.7	5.70	119.7^{ab}	4.22	108.2^{a}	132.6^{a}
[60]	Betaine 10	74.2	34.9	39.3	0.90	94.2	5.08	1077.3	5.15	107.3^{a}	4.10	107.8^{a}	134.3^{a}
	Betaine 20	76.6	34.3	42.4	0.81	343.9	5.14	770.5	4.80	132.9^{ab}	4.22	108.2^{a}	135.5^{a}
	Betaine 40	78.5	35.2	43.2	0.81	94.1	5.01	963.8	5.95	136.7^{b}	4.51	108.4^{a}	134.5^{a}
	Glycerol	74.7	35.2	39.5	0.91	172.5	5.08	982.7	4.63	115.8^{ab}	4.16	107.4^{a}	133.8^{a}
	SE	1.5	0.7	1.5	0.04	379.9	0.21	83.9	0.45	10.3	0.15	3.9	2.0
February	Control	71.9	35.4	36.5	0.97	109.1	5.18	1073.5	7.33	117.5^{ab}	4.00	109.2^{a}	132.1^{b}
[90]	Betaine 10	71.2	33.6	37.5	0.90	86.4	4.99	111.9	6.74	113.3^{a}	3.92	107.8^{a}	131.8^{b}
	Betaine 20	74.0	35.0	39.1	0.91	190.3	5.09	1095.3	6.96	127.6^{ab}	3.99	108.2^{a}	131.6^{b}
	Betaine 40	73.7	34.7	38.9	0.89	106.3	5.04	1124.1	7.21	136.7^{b}	4.10	107.5^{a}	131.8^{b}
	Glycerol	74.2	35.3	38.8	0.92	222.0	5.03	1196.2	6.61	119.0^{ab}	3.85	108.7^{a}	141.0^{a}
	SE	1.3	0.6	1.3	0.04	314.7	0.19	71.0	0.38	8.8	0.12	3.2	1.7
March	Control	71.3	35.3	36.1	0.99	118.0	5.08	1058.0	6.86	116.8^{a}	4.07	109.2^{a}	134.0^{a}
[110]	Betaine 10	71.3	34.3	37.0	0.93	128.8	4.73	1187.8	6.38	115.8^{a}	4.07	109.5^{a}	134.2^{a}
	Betaine 20	73.6	35.3	38.3	0.93	144.5	5.02	1148.3	6.45	118.8^{a}	4.01	109.1^{a}	135.3^{a}

	Betaine 40	72.9	35.3	37.6	0.94	111.3	5.02	1185.8	6.64	127.5[a]	4.01	108.9[a]	134.9[a]
	Glycerol	74.5	35.0	39.5	0.90	267.8	4.98	1166.6	6.55	125.0[a]	4.23	110.8[a]	133.1[a]
	SE	1.0	0.4	1.02	0.03	215.3	0.15	50.6	0.27	6.39	0.08	2.1	1.2
F-prob	Diet (D)	0.17	0.05	0.06	0.10	0.44	0.88	0.48	0.17	0.05	0.49	0.15	0.9
	Month (M)	<0.001	<0.001	<0.001	<0.001	0.007	<0.001	<0.001	<0.001	<0.001	<0.001	0.06	<0.001
	D × M	0.82	0.27	0.54	0.18	0.06	0.66	0.52	0.88	0.04	0.17	0.03	<0.001

[A] Creatine phosphokinase
[B] Lactate dehydrogenase
[C] Globulin
[D] Albumin to Globulin ratio
[E] Creatine phosphokinase
[F] Lactate dehydrogenase
[G] Creatinine
Standard error (SE) is presented as a pooled value for individual parameters (columns) within each month.
Means within a column for each month with superscripts are significantly different ($P < 0.05$).

Table 30 Effect of shade (no shade and shade) within month on plasma concentration of heat shock protein 70 (hsp70) and blood haematological parameters, sampled from steers with temperature transmitters at 0 (induction), 30, 60, 90 or 110 days on feed (DOF)

Month [DOF]	Shade	Hsp70 (OD)	WBC[A] (x10e9/L)	Neutrophil (N) (%)	Lymphocyte (L) (%)	N:L (%)	Monocyte (%)	Eosinophil (%)	Basophil (%)	RBC[B] (x10e12/L)	HGB[C] (g/L)	HCT[D] (%)	Platelets (x10e9/L)
November	Nil shade	0.2	11.4	27.6	62.9	0.52	6.86	0.92	1.81	7.06	106.5	25.4	176.5
[0]	Shade	0.18	11.1	28.6	62.1	0.57	6.70	0.78	1.76	7.36	113.1	26.3	158.2
	SE	0.12	0.6	2.2	2.7	0.04	0.69	0.32	2.57	0.24	3.7	0.7	3.4
December	Nil shade	0.9	11.9	23.3	67.8	0.42	6.44	1.34	1.10	6.13	112.9	26.0	312.5
[30]	Shade	0.96	10.0	24.7	65.0	0.43	7.99	1.22	1.32	6.12	112.3	26.4	335.9
	SE	0.17	0.9	3.2	3.9	0.07	1.05	0.49	3.95	0.31	4.6	0.9	50.0
January	Nil shade	0.83	12.5	17.0	76.8	0.16	3.85	1.50	1.29	7.15	130.0	30.1	165.1
[60]	Shade	0.78	12.1	18.1	73.2	0.27	4.67	2.49	1.61	6.80	125.5	29.1	254.7
	SE	0.19	1.0	3.7	4.5	0.08	1.25	0.58	4.75	0.34	5.0	1.0	58.9
February	Nil shade	0.72	10.5	26.1	62.7	0.51	5.76	3.05	8.89	7.83	134.9	30.7	265.9
[90]	Shade	0.49	10.5	27.0	61.2	0.48	7.43	2.37	8.81	7.42	136.2	29.7	207.1
	SE	0.17	0.9	3.2	3.9	0.06	1.05	0.49	3.95	0.31	4.6	0.9	50.0
March	Nil shade	0.59	10.2	24.2	68.8	0.40	4.27	1.63	2.04	8.20	136.8	31.5	258.5
[110]	Shade	0.32	10.1	29.1	63.0	0.58	3.87	2.34	1.75	8.08	138.1	31.7	229.1
	SE	0.12	0.6	2.2	2.7	0.04	0.68	0.32	2.55	0.24	3.6	0.7	33.1
F-prob	Shade (S)	0.53	0.50	0.50	0.40	0.45	0.35	0.67	0.99	0.71	0.91	0.91	1.00
	Month (M)	<0.001	0.002	<0.001	<0.001	0.005	<0.001	<0.001	0.1607	<0.001	<0.001	<0.001	<0.001
	S × M	0.49	0.74	0.83	0.83	0.68	0.70	0.31	1.00	0.37	0.45	0.46	0.68

[A] White Blood Cells
[B] Red Blood Cells
[C] Haemoglobin
[D] Haematocrit
Standard error (SE) is presented as a pooled value for individual parameters (columns) within each month.

Table 31 Effect of shade (no shade and shade) within month on plasma concentration of biochemistry parameters, sampled from steers with temperature transmitters at 0 (induction), 30, 60, 90 or 110 days on feed (DOF)

Month	Shade	Prot[A] g/L	Alb[B] ng/L	Glo[C] g/L	Alb:Glo[D] ratio	CPK[E] U/L	Glucose umol/L	LDH[F] U/L	Urea umol/L	Creat[G] umol/L	K mmol/L	Cl mmol/L	Na mmol/L
November	Nil shade	72.7	34.9	37.8	0.93	620.8	4.81	901.6	3.44	161.2	4.47	106.0[a]	131.1[a]
[0]	Shade	73.2	35.4	37.8	0.94	54.3	4.62	903.6	3.36	156.0	4.37	105.6[a]	130.5[a]
	SE	0.6	0.3	0.7	0.02	140.3	0.10	32.9	0.17	4.1	0.05	1.4	0.8
December	Nil shade	73.5	33.8	39.7	0.86	155.0	5.02	825.2	5.56	143.7	4.21	100.7[b]	134.1[a]
[30]	Shade	73.6	34.5	39.0	0.90	217.0	4.92	893.5	5.71	126.8	4.45	110.9[a]	129.4[b]
	SE	0.9	0.4	0.9	0.02	21.6	0.13	47.6	0.25	5.9	0.08	2.1	1.1
January	Nil shade	77.1	35.4	41.7	0.85	169.1	5.19	935.1	5.13	131.3	4.33	109.0[a]	131.7[b]
[60]	Shade	74.6	34.9	39.6	0.89	161.0	5.01	946.9	5.36	113.6	4.15	107.1[a]	136.5[a]
	SE	1.0	0.4	1.0	0.03	246.8	0.14	54.4	0.29	6.7	0.10	2.6	1.3
February	Nil shade	72.6	34.6	38.0	0.92	96.8	5.08	1049.9	6.72	129.4	4.01	108.1[a]	128.9[b]
[90]	Shade	73.4	35.0	38.3	0.92	188.9	5.05	1190.5	7.22	116.3	3.94	108.4[a]	138.5[a]
	SE	0.9	0.4	0.9	0.02	207.8	0.12	46.8	0.25	5.8	0.08	2.1	1.1
March	Nil shade	72.6	34.8	37.7	0.93	124.7	4.95	1079.9	6.26	121.9	4.05	109.3[a]	134.8[a]
[110]	Shade	72.8	35.2	37.6	0.94	183.5	4.98	1218.6	6.89	119.6	4.11	109.7[a]	133.8[a]
	SE	0.6	0.3	0.6	0.02	136.1	0.10	32.0	0.17	4.0	0.05	1.4	0.7
F-prob	Shade (S)	0.80	0.40	0.50	0.39	0.91	0.50	0.11	0.19	0.11	0.87	0.20	0.12
	Month (M)	<0.001	<0.001	<0.001	<0.001	0.007	<0.001	<0.001	<0.001	<0.001	<0.001	0.06	<0.001
	S × M	0.28	0.64	0.53	0.74	0.98	0.40	0.09	0.16	0.39	0.06	0.04	<0.001

[A] Total Protein
[B] Albumin
[C] Globulin
[D] Albumin to Globulin ratio
[E] Creatine phosphokinase
[F] Lactate dehydrogenase
[G] Creatinine
Standard error (SE) is presented as a pooled value for individual parameters (columns) within each month.
Means within a column for each month with superscripts are significantly different ($P < 0.05$).

4.15 Animal response during defined 'hot' periods of the study

The climatic data was analysed to identify a number of hot periods during the study. The HLI calculated for a specific 34 day period of the study is shown in Figure 6.

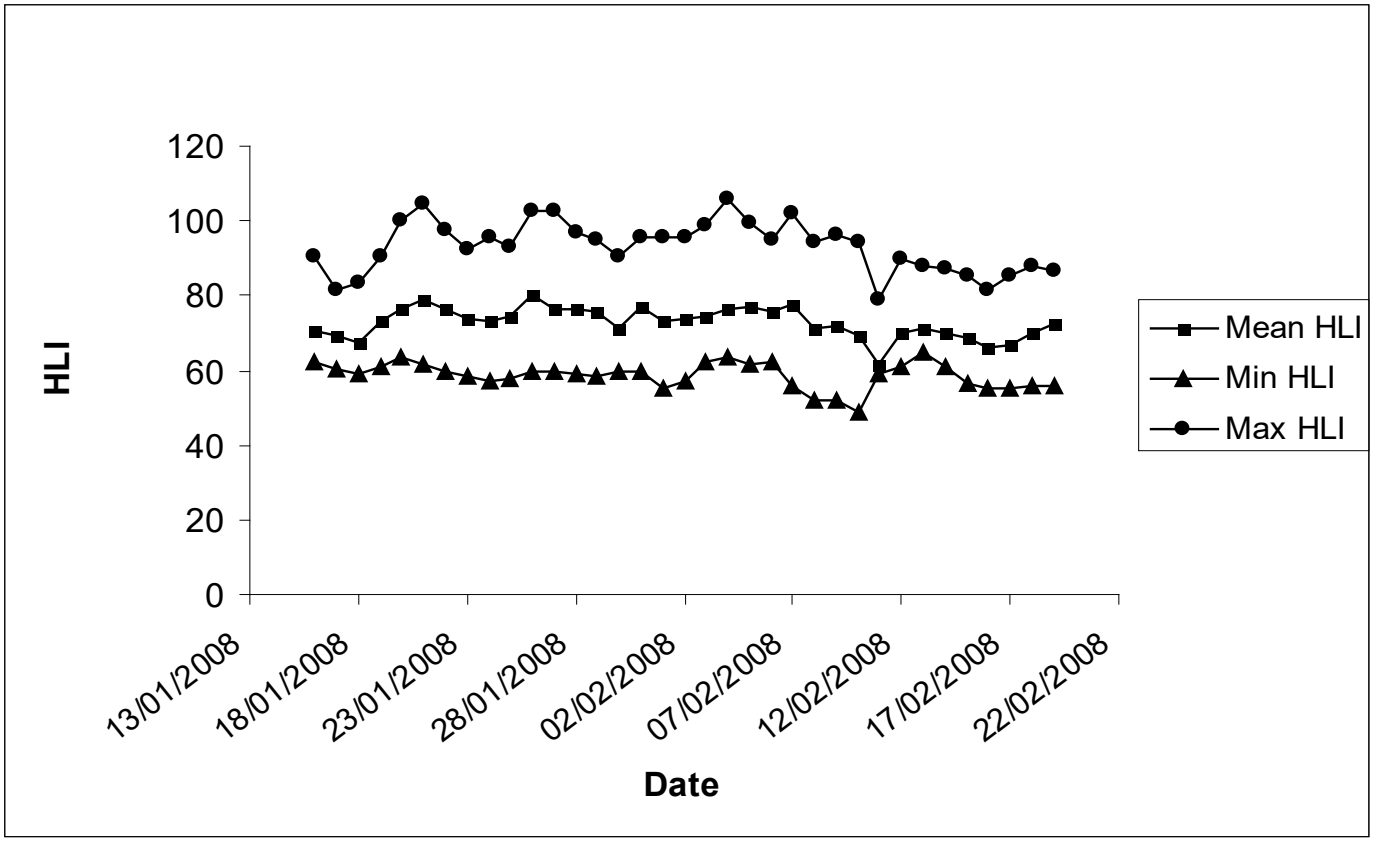

Figure 6 Minimum, mean and maximum HLI recorded over a 34 day period from 16 January 2008 to 19 February 2008

The AHLU calculated for a specific 34 day period of the study is shown in Figure 7. The most prolonged period of high AHLU values were recorded from January 20 to February 10, 2008.

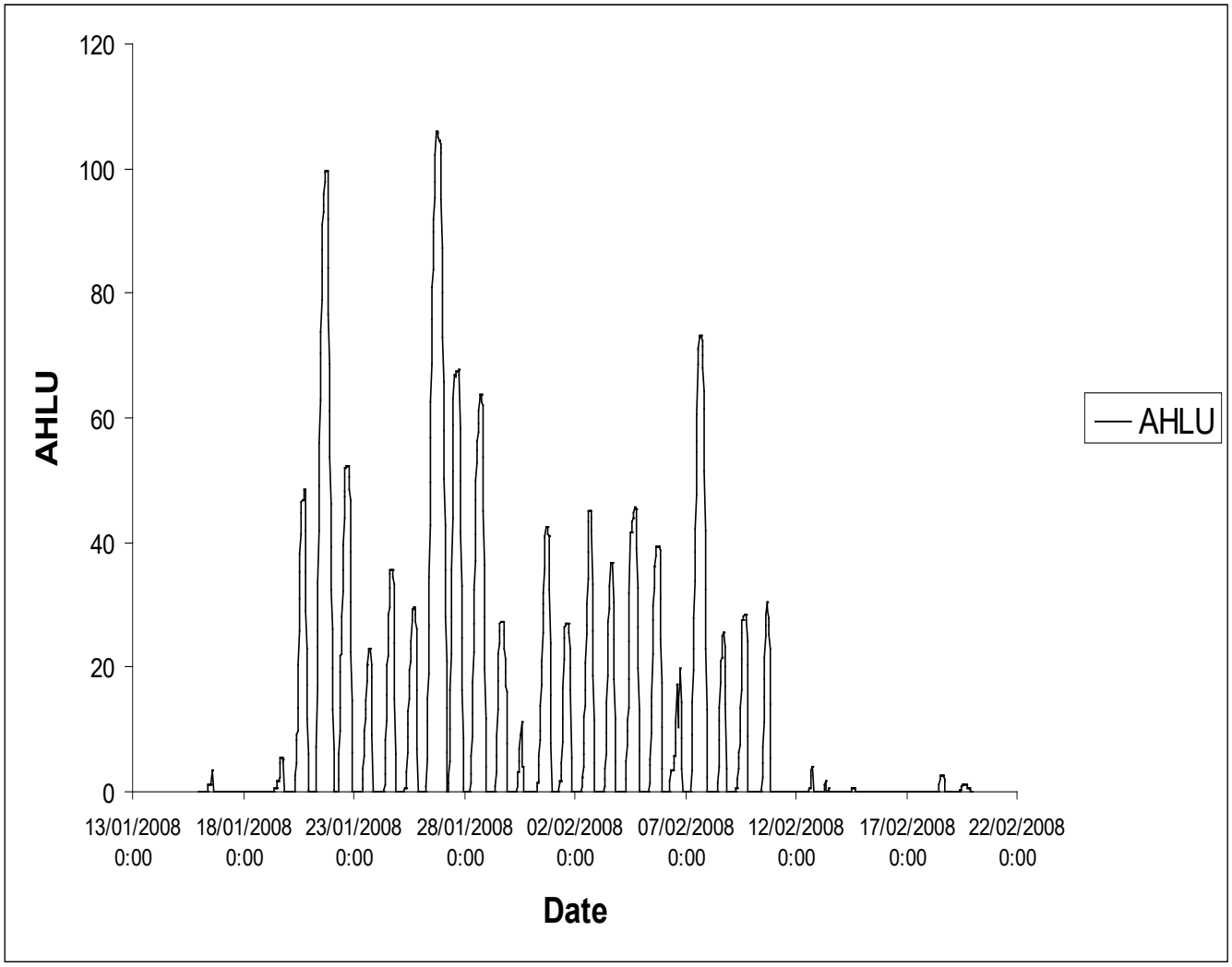

Figure 7 The AHLU recorded over a 34 day period from 16 January, 2008 to 19 February, 2008

Based on key climatic determinants of HLI and AHLU, a number of periods likely to induce heat stress were identified during a 34 day period from 16 January 2008 to 19 February, 2008. These periods coincided with a number of high heat load events, with some such events being sequential.

Dietary treatment influenced DMI ($P<0.05$) on a number of days during the 34 day period from 16 January, 2008 to 19 February, 2008 (Figure 8).

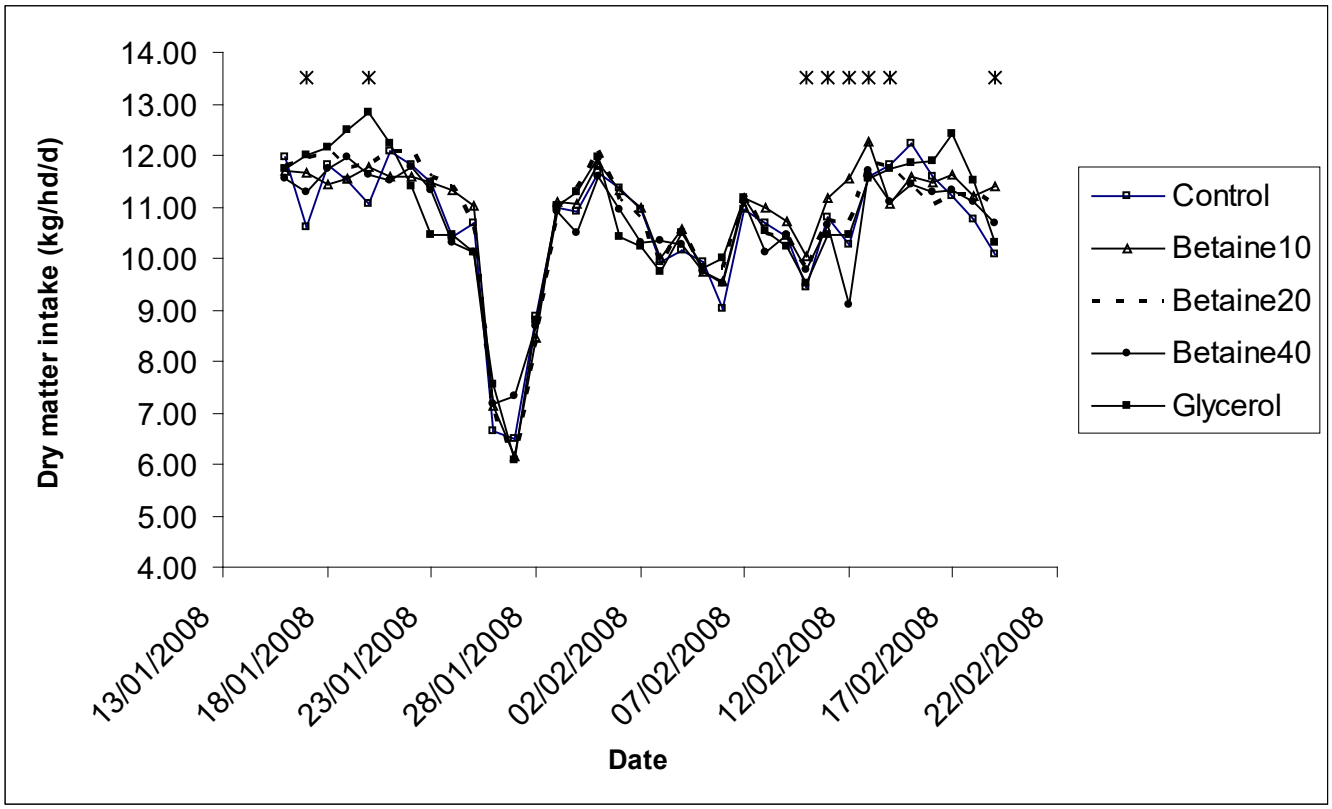

Figure 8 Effect of diet on the DMI of steers over a 34 day period which includes defined hot periods. (* indicates days where dietary treatments were different (P<0.05))

The days were dietary differences (P<0.05) in DMI were recorded are shown in Table 32. In general, Betaine 10 resulted in higher DMI, apart from 14 February where it resulted in a lower DMI (P<0.05). There was no other consistent pattern of dietary effect on these days.

The January days where differences were recorded, were of low AHLU, while the February days of interest were of higher AHLU, but not considered excessively high (Figure 7), thus they were days where the high load was not significant. For these days of marginal heat load, diet may have had a more intrinsic/direct effect on DMI and not an indirect effect of ameliorating heat load.

Table 32 Effect of diet (Betaine 10 (10 g/steer/d), 20 (20 g/steer/d) or 40 (40 g/steer/d)) or glycerol (5% DMB) on the DMI of steers on days during a 34 day period where differences were recorded

	Date							
	17/01/2008	20/01/2008	10/02/2008	11/02/2008	12/02/2008	13/02/2008	14/02/2008	19/02/2008
Control	10.62^{a}	11.07^{a}	9.43^{a}	10.79^{b}	10.26^{b}	11.60^{ab}	11.81^{b}	10.09^{a}
Betaine 10	11.66^{b}	11.79^{b}	10.05^{d}	11.15^{c}	11.53^{c}	12.26^{c}	11.07^{a}	11.40^{c}
Betaine 20	11.96^{b}	11.82^{b}	9.73^{bc}	10.75^{b}	10.68^{b}	11.90^{b}	11.77^{b}	11.05^{bc}
Betaine 40	11.28^{ab}	11.63^{b}	9.79^{c}	10.65^{ab}	9.10^{a}	11.69^{ab}	11.10^{a}	10.69^{ab}
Glycerol	11.99^{b}	12.82^{c}	9.51^{ab}	10.46^{a}	10.46^{b}	11.55^{a}	11.74^{b}	10.29^{a}
SE	0.29	0.17	0.08	0.09	0.25	0.10	0.20	0.21

Means within a column within an effect with different superscripts are significantly different (*P < 0.05*). SE = standard error.

The provision of shade influenced DMI (P<0.05) on a number of days during the 34 day period from 16 January, 2008 to 19 February, 2008 (Figure 9). The DMI was higher (P<0.05) in the shaded pens on 16, 20, 21, 23 and 25 to 29 January and 3, 4, 8 to 10 and 16 February, 2008. The results indicate a direct response of shade on DMI during these days.

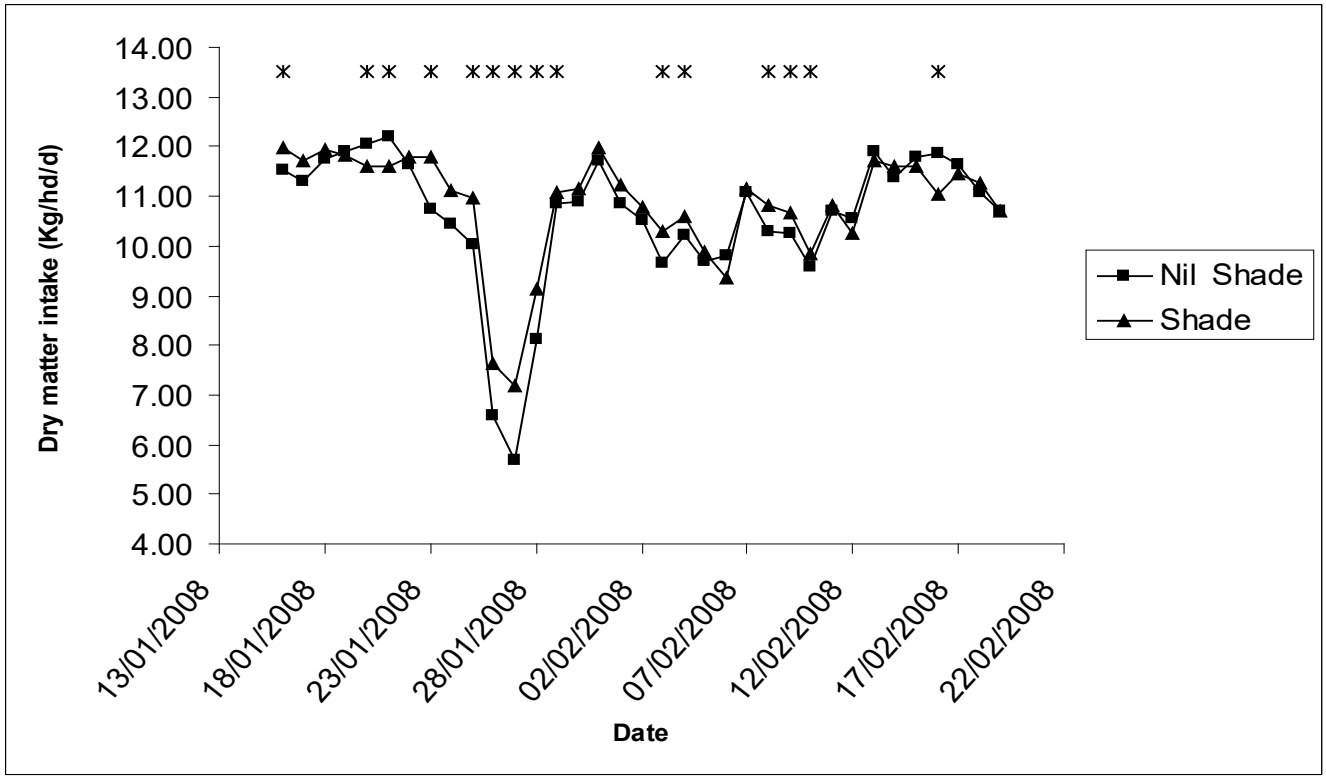

Figure 9 Effect of shade on the DMI of steers over a 34 day period which includes defined hot periods. (* indicates days where shade treatments were different (P<0.05))

The diet × shade interaction (P<0.05) on the DMI of steers on a number of days during the 34 day period from 16 January, 2008 to 19 February, 2008 is shown in Figure 10.

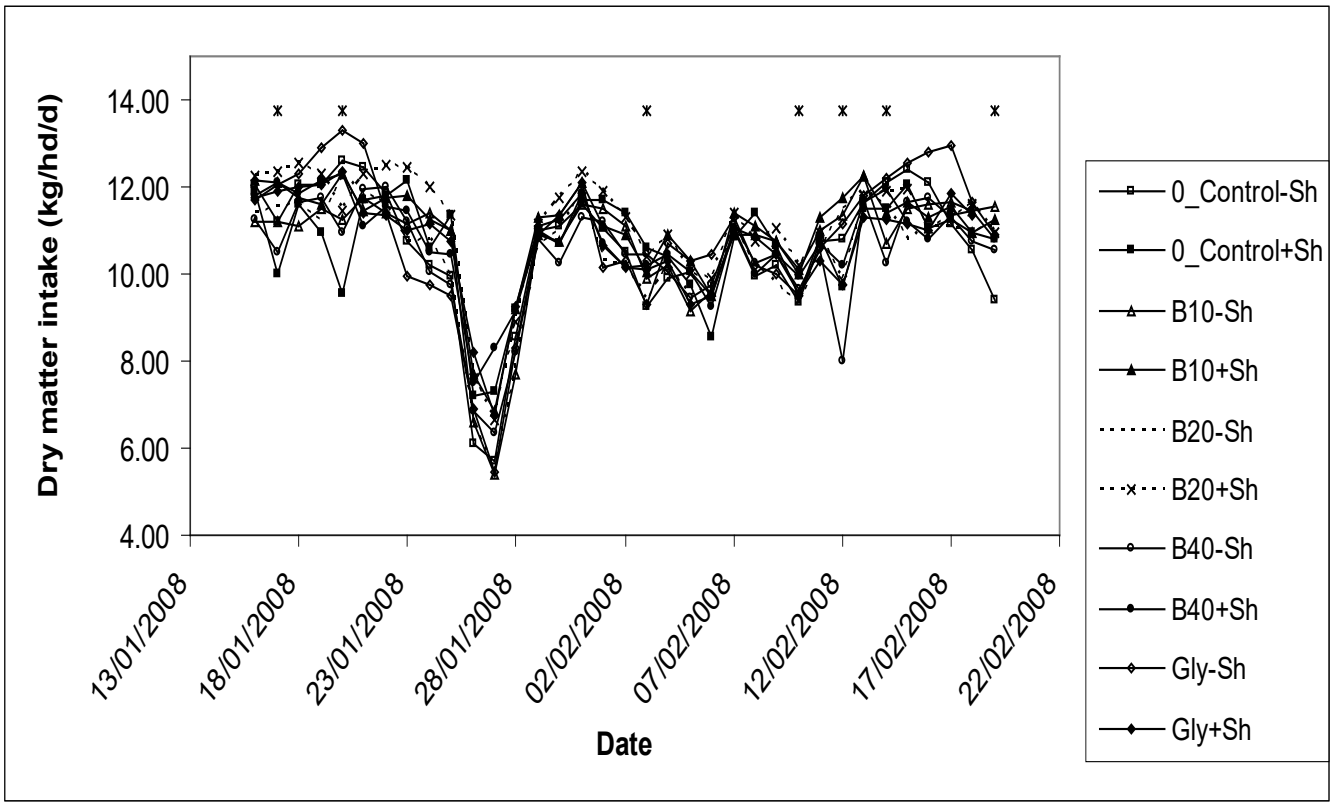

Figure 10 Diet × shade interaction of DMI of steers over a 34 day period which includes defined hot periods. (* indicates days where the diet*shade interaction were recorded (P<0.05)

The actual days where a diet × shade interaction (P<0.05) on the DMI of steers was recorded are shown in Table 33. In general, the interaction appears to be due to the switch in order of DMI magnitude due to treatment on certain days i.e. normally high DMI treatments recording low DMI and the converse.

Table 33 The days on which a diet × shade interaction on DMI of steers was recorded

	Date						
	17/01/2008	20/01/2008	03/02/2008	10/02/2008	12/02/2008	14/02/2008	19/02/2008
Control-Nil Shade	11.21^{ab}	12.58^{a}	9.26^{b}	9.38^{c}	10.82^{a}	12.10^{ab}	9.39^{d}
Control-Shade	10.03^{b}	9.56^{e}	10.62^{a}	9.48^{c}	9.70^{c}	11.52^{abc}	10.80^{ab}
Betaine 10-Nil Shade	11.23^{ab}	11.26^{d}	9.91^{a}	10.10^{a}	11.33^{ab}	10.72^{cd}	11.56^{a}
Betaine 10-Shade	12.10^{a}	12.33^{b}	10.07^{a}	10.00^{a}	11.73^{a}	11.42^{abc}	11.25^{ab}
Betaine 20-Nil Shade	11.54^{a}	12.19^{bc}	9.39^{ab}	9.26^{d}	11.49^{a}	11.62^{ab}	11.15^{ab}
Betaine 20-Shade	12.37^{a}	11.46^{cd}	10.42^{a}	10.20^{a}	9.88^{c}	11.92^{ab}	10.94^{ab}
Betaine 40-Nil Shade	10.48^{ab}	10.96^{d}	10.47^{a}	9.63^{bc}	8.00^{d}	10.27^{d}	10.56^{bc}
Betaine 40-Shade	12.08^{a}	12.30^{b}	10.21^{a}	9.95^{ab}	10.21^{b}	11.93^{ab}	10.83^{ab}
Glycerol-Nil Shade	12.06^{a}	13.31^{a}	9.33^{ab}	9.49^{c}	11.16^{ab}	12.23^{a}	10.92^{ab}
Glycerol-Shade	11.92^{a}	12.33^{b}	10.11^{a}	9.53^{c}	9.76^{b}	11.25^{b}	9.67^{cd}
SE	0.41	0.25	0.24	0.11	0.36	0.28	0.29

Means within a column within an effect with different superscripts are significantly different (*P < 0.05*). SE = standard error

Dietary treatment influenced water consumption (P<0.05) on a number of days during the 34 day period from 16 January, 2008 to 19 February, 2008 (Figure 11).

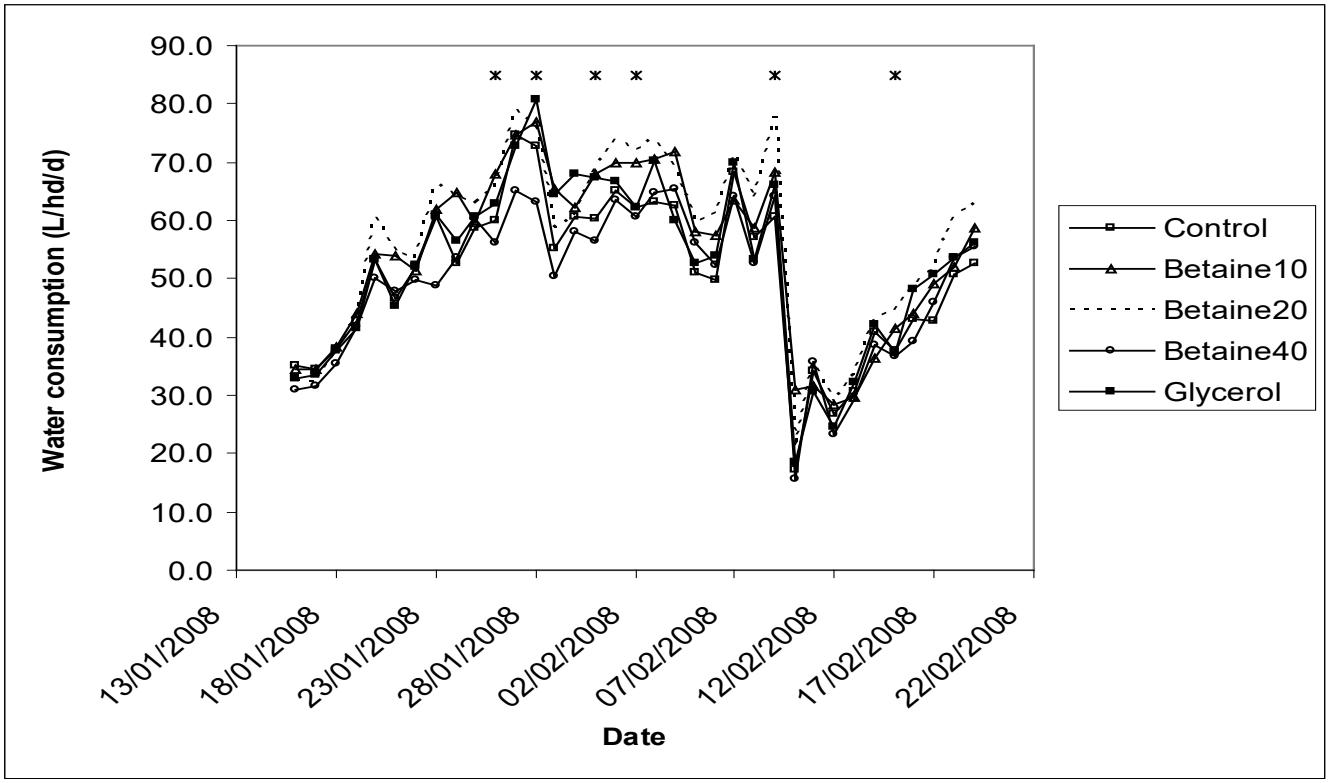

Figure 11 Effect of diet on the water consumption of steers over a 34 day period which includes defined hot periods. (* indicates days where dietary treatments were different (P<0.05))

The days were dietary differences (P<0.05) were recorded are shown in Table 34 In general, on a number of days, Betaine 10 and Betaine 20 resulted in higher water consumption (P<0.05). On some days (26 and 28 January, 2008), both the Control and Glycerol treatments resulted in a similar water consumption to Betaine 10 and Betaine 20.

The January days where differences were recorded, were of moderate to high AHLU, while the February days of interest were of low to moderate AHLU, (Figure 7). Thus the higher water consumption values of 26 and 28 January, 2008 are not unexpected. None of the days where higher DMI were recorded by the Betaine 10 treatment, and the days were higher water consumption was recorded by the same treatment, actually coincided or occurred on days either just prior or just after.

Table 34 Effect of diet (Betaine 10 (10 g/steer/d), 20 (20 g/steer/d) or 40 (40 g/steer/d)) or glycerol (5% DMB) on the water consumption of steers on days during a 34 day period where differences were recorded

	Date					
	26/01/2008	28/01/2008	31/01/2008	02/02/2008	09/02/2008	15/02/2008
Control	59.9^{b}	72.8^{ab}	60.5^{ab}	62.3^{bc}	60.6^{b}	37.5^{b}
Betaine 10	68.0^{a}	76.8^{a}	68.0^{a}	69.9^{ab}	68.3^{ab}	41.6^{ab}
Betaine 20	65.9^{a}	75.7^{a}	69.2^{a}	72.1^{a}	77.7^{a}	44.7^{a}
Betaine 40	56.2^{b}	63.1^{b}	56.6^{b}	60.6^{c}	64.0^{b}	36.5^{b}
Glycerol	62.9^{ab}	80.7^{a}	67.2^{a}	62.2^{bc}	66.1^{b}	37.2^{b}
SE	2.5	3.3	2.6	2.7	3.4	1.7

Means within a column within an effect with different superscripts are significantly different (*P <0.05*). SE = standard error

The provision of shade influenced water consumption (P<0.05) on a number of days during the 34 day period from 16 January, 2008 to 19 February, 2008 (Figure 12). Water consumption was higher (P<0.05) in the shaded pens for the periods 22 to 24 January, 26 January to 3 February and 7 to 9 February, 2008. The results indicate a direct response of shade on water consumption during these days.

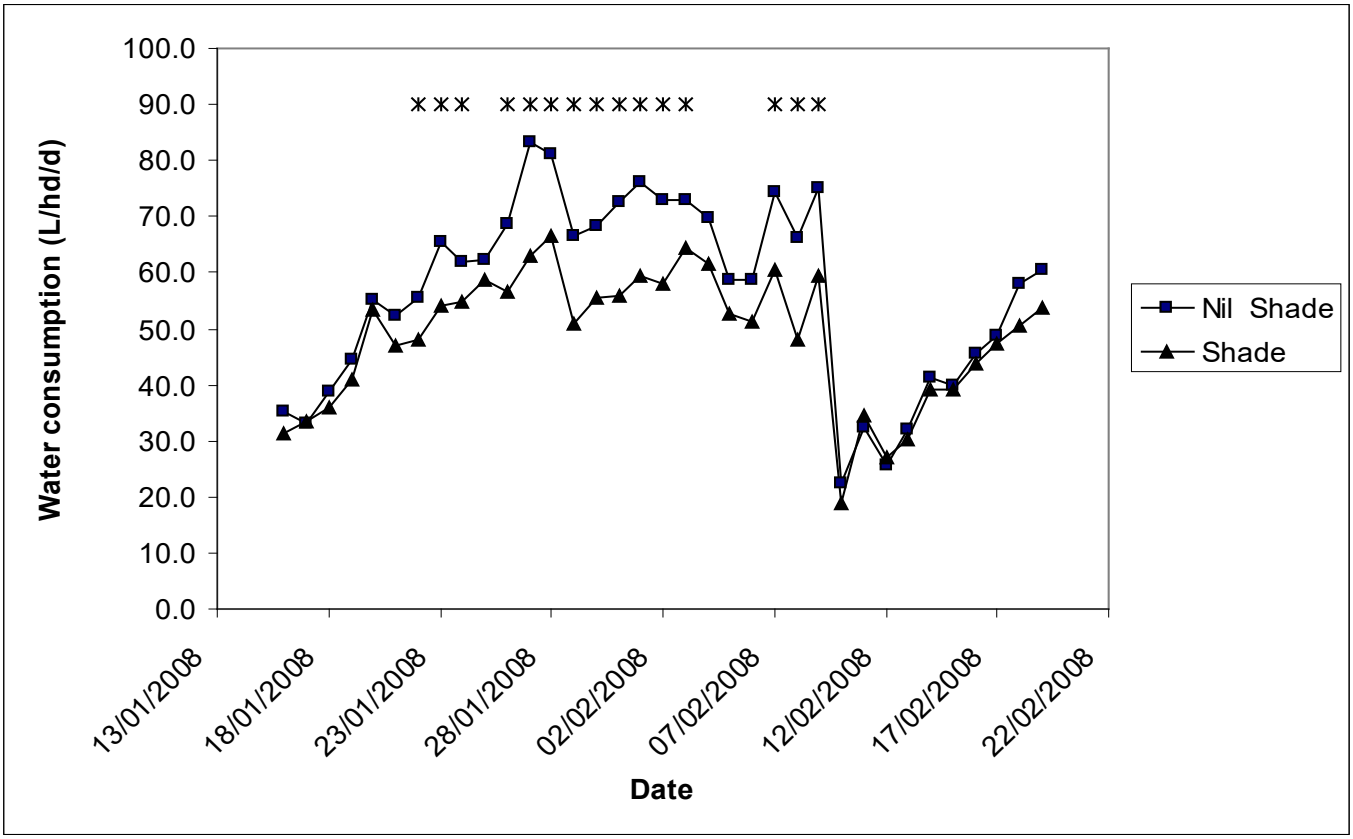

Figure 12 Effect of shade on the water consumption of steers over a 34 day period which includes defined hot periods. (* indicates days where shade treatments were different (P<0.05))

The diet × shade interaction (P<0.05) recorded on one day of the 34 day period which included defined hot periods from 16 January, 2008 to 19 February, 2008 is shown in Figure 13.

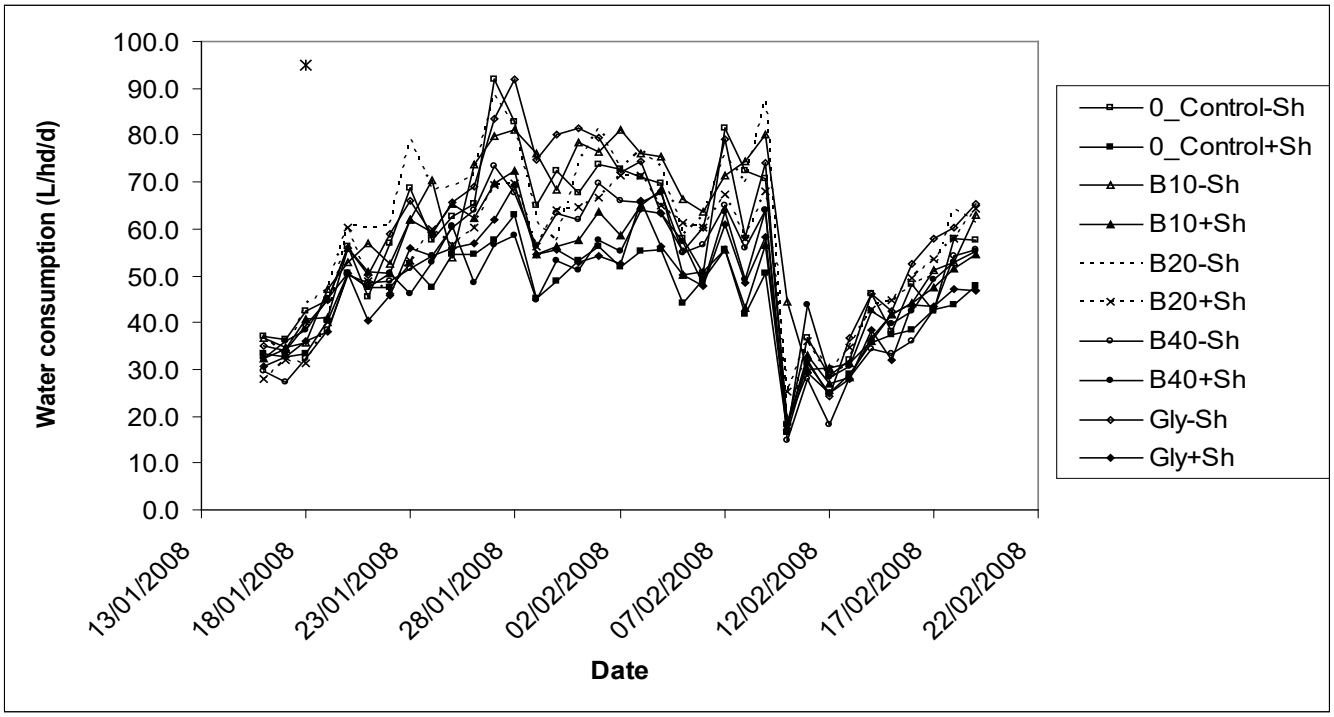

Figure 13 Diet × shade interaction of water consumption of steers over a 34 day period which includes defined hot periods. (* indicates days where the diet × shade interaction was recorded (P<0.05))

The actual diet × shade interaction (P<0.01) recorded on steer water consumption on 18 January, 2008 is shown in Table 35. The interaction is a result of the increased water consumption in the shaded pens of the Betaine 10 (not significant) and Betaine 40 (P<0.05) dietary treatments in contrast to all other dietary treatments where water consumption decreased significantly (Control and Betaine 20) due to shade.

Table 35 Diet × shade interaction on water consumption of steers on 18 January 2008

	18/01/2008
Control-Nil Shade	42.5^{ab}
Control-Shade	33.5^{cd}
Betaine 10-Nil Shade	35.6^{bc}
Betaine 10-Shade	40.9^{ab}
Betaine 20-Nil Shade	43.7^{a}
Betaine 20-Shade	31.5^{de}
Betaine 40-Nil Shade	32.3^{d}
Betaine 40-Shade	38.3^{abc}
Glycerol-Nil Shade	39.5^{abc}
Glycerol-Shade	35.9^{bc}
SE	2.3

Means within the column within an effect with different superscripts are significantly different (*P < 0.05*).

Dietary treatment had no influence (P>0.05) on mean, maximum or minimum body temperature over the 34 day period which includes defined hot periods.

The provision of shade reduced the mean body temperature of steers with implanted temperature transmitters (P<0.05) on a number of days over a 34 days period which included defined hot periods from 16 January, 2008 to 19 February, 2008. Specifically, these days were: 17, 21, 22, 24 to 26, 28, 29 January, 2008 and from 11 to 14 February 2008 (Figure 14). Over these days, the maximum reduction in mean body temperature was 0.45 °C. When compared to the data of Figure 7, these days varied from zero AHLU (12 to 14 February), low AHLU (24 and 29 January), moderate AHLU (22 and 28 January) to high AHLU (21 and 26 January (AHLU of 105), 2008). Thus, there were a number of days during this period when shade reduced body temperature that AHLU was not considered excessive, however the response due to shade was marked.

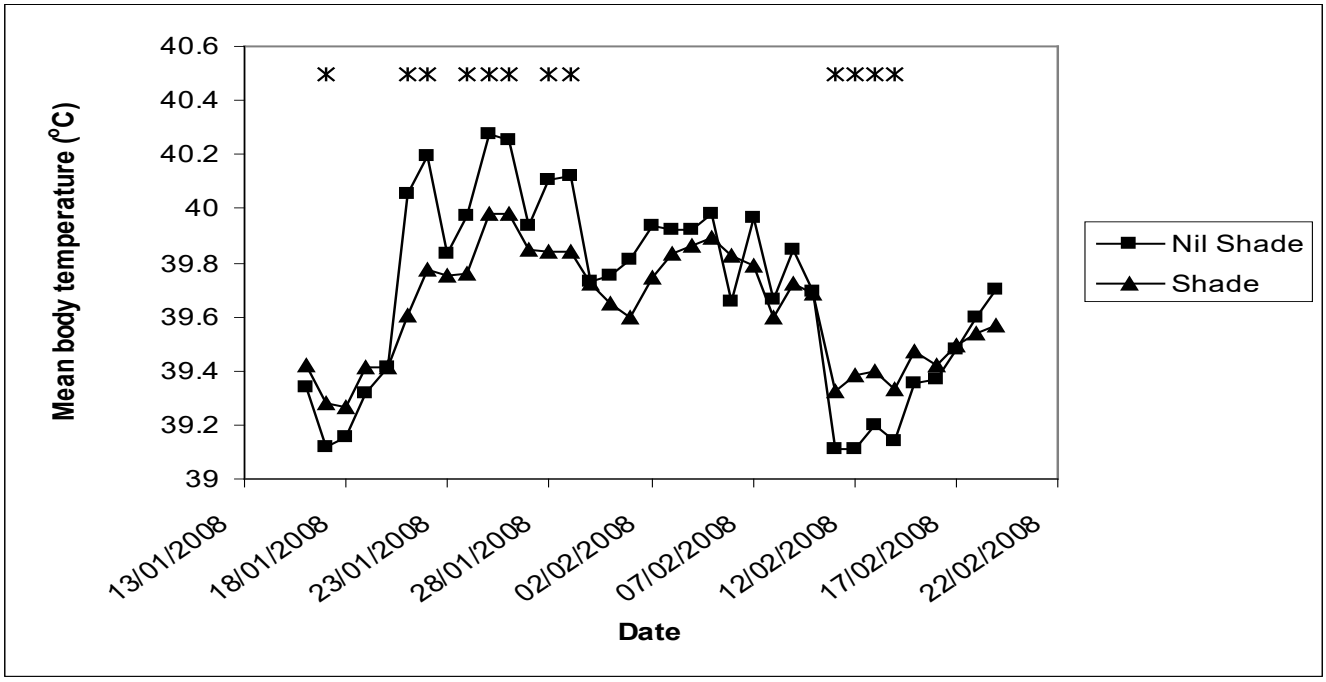

Figure 14 Effect of shade on the mean body temperature (°C) of steers with implanted temperature transmitters over a 34 day period which includes defined hot periods. (* indicates days where shade treatments were different (P<0.05))

The provision of shade reduced the range in body temperature of steers implanted with temperature transmitters (P<0.05) on a number of days over a 34 days period which included defined hot periods from 16 January, 2008 to 19 February, 2008 (Figure 15). Specifically, shade reduced the range in body temperature on 26 days out of the 34 day period, with no difference being recorded due to shade on 16 to 20 January and 11 to 13 February, 2008. Over these 26 days, the maximum reduction in range in body temperature was 1.36 °C. While Figure 7 has shown a wide variation in AHLU over this 34 day period from zero AHLU to high values over 100, the marked benefit to shade in reducing the range in body temperature is apparent.

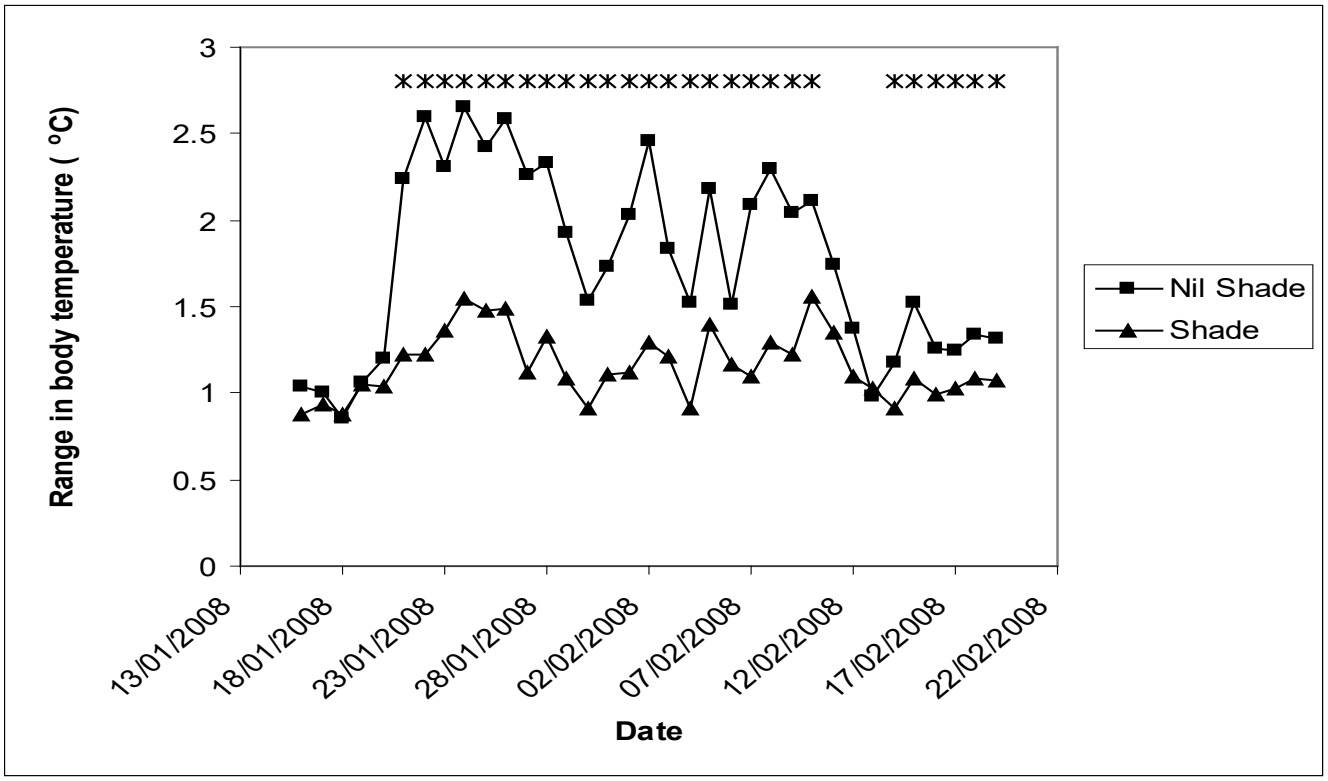

Figure 15 Effect of shade on the range in body temperature (°C) of steers with implanted temperature transmitters over a 34 day period which includes defined hot periods. (* indicates days where shade treatments were different (P<0.05))

The provision of shade reduced the maximum body temperature of steers with implanted temperature transmitters (P<0.05) on a number of days over a 34 day period which included defined hot periods from 16 January, 2008 to 19 February, 2008 (Figure 16). Specifically, shade reduced the maximum body temperature on 22 days out of the 34 day period, with no difference being recorded due to shade on 16 to 20 January and 6, 11, and 14 to 18 February, 2008. Over these 22 days, the maximum reduction in maximum body temperature was 1.30 °C. As the per the data for mean body temperature and the range in body temperature, the impact of shade in reducing maximum body temperature was marked on many days regardless of the climatic conditions on the 'day' during this 34 day period.

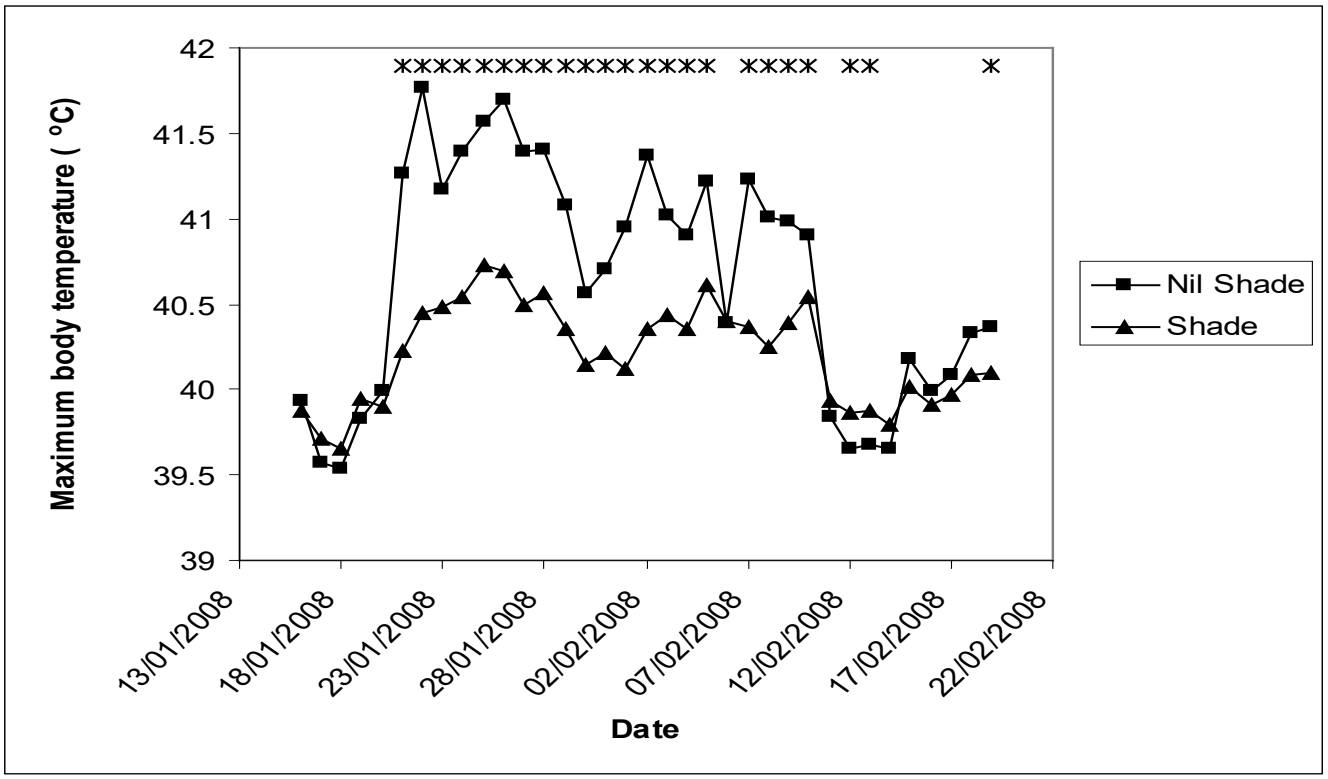

Figure 16 Effect of shade on maximum body temperature (°C) of steers with implanted temperature transmitters over a 34 day period which includes defined hot periods. (* indicates days where shade treatments were different (P<0.05))

The provision of shade increased the minimum body temperature of steers with implanted temperature transmitters (P<0.05) on a number of days over a 34 days period which included defined hot periods from 16 January 2008 to 19 February 2008 (Figure 17). The minimum body temperature of the shaded steers was higher than the non-shaded steers on the 23, 24, 27 and 30 January and 6, 8, 12, and 14 February, 2008. Note that in many cases, these days immediately followed the days where a reduced maximum temperature due to shade was recorded (Figure 16). Over these days, the maximum increase in minimum body temperature was 0.40 °C. Thus in contrast to reducing body temperature, the provision of shade increased the minimum body temperature on some days during the 34 day period. As a consequence, it could be suggested that steers under feedlot shade may be more thermoneutral during the conditions described over this 34 day period i.e. less marked diurnal variation in body temperature due to a slightly higher minimum body temperature in conjunction with a reduced maximum body temperature.

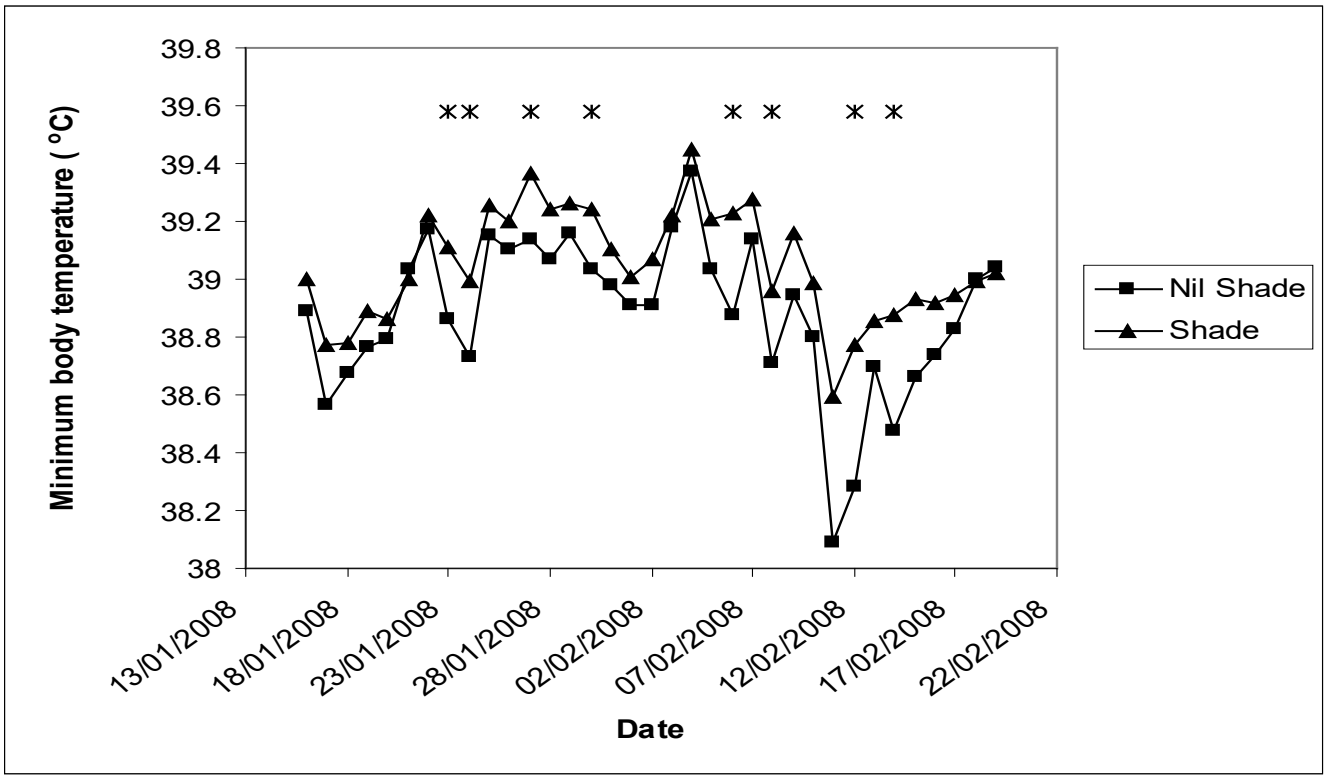

Figure 17 Effect of shade on minimum body temperature (°C) of steers with implanted temperature transmitters over a 34 day period which includes defined hot periods. (* indicates days where shade treatments were different (P<0.05))

Dietary treatment influenced midday panting (P<0.05) scores on 16, 18 and 19 February 2008 (Figure 18) during the 34 day period with defined hot periods from 16 January to 19 February, 2008.

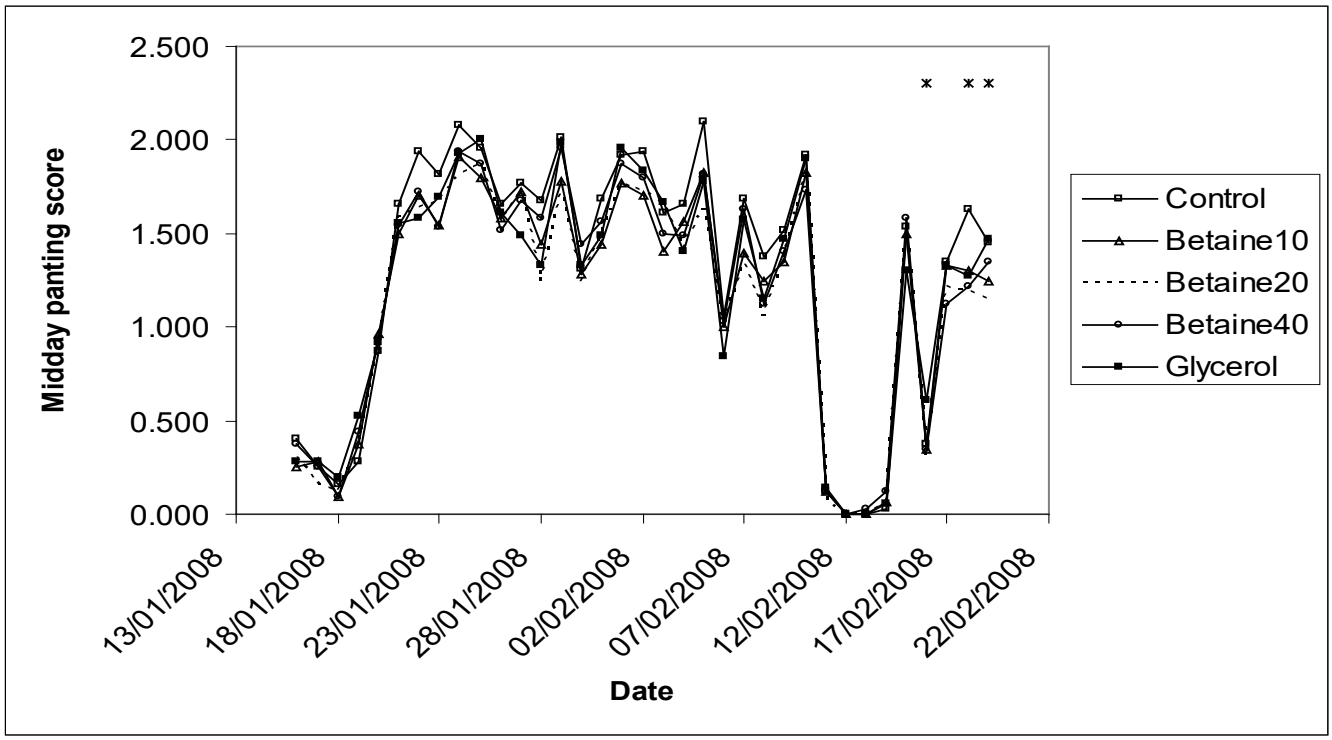

Figure 18 Effect of diet on the midday panting scores of steers over a 34 day period which includes defined hot periods. (* indicates days where dietary treatments were different (P<0.05))

The actual days were dietary differences in midday panting scores (P<0.05) were recorded are shown in Table 36. The Control and Glycerol treatments resulted in higher midday panting scores (P<0.05) with Glycerol highest on 16 February, Control highest on 18 February and both Control and Glycerol highest on 19 February. It is unknown as to why these treatments resulted in higher midday panting scores, as these dietary treatments had no influence on body temperature on these days.

Table 36 Effect of diet (Betaine 10 (10 g/steer/d), 20 (20 g/steer/d) or 40 (40 g/steer/d)) or glycerol (5% DMB) on the midday panting score of steers on days during a 34 day period where differences were recorded

	Date		
	16/02/2008	18/02/2008	19/02/2008
Control	0.375[b]	1.625[a]	1.453[a]
Betaine 10	0.344[b]	1.297[b]	1.250[b]
Betaine 20	0.313[b]	1.188[b]	1.156[b]
Betaine 40	0.344[b]	1.219[b]	1.344[b]
Glycerol	0.611[a]	1.278[b]	1.472[a]
SE	0.042	0.066	0.067

Means within a column within an effect with different superscripts are significantly different (*P < 0.05*). SE = standard error

The provision of shade reduced the midday panting score (P<0.05) of steers over 27 of the 34 days with defined hot periods from 16 January to 19 February 2008 (Figure 19). The maximum reduction in midday panting score on any one day was 1.183.

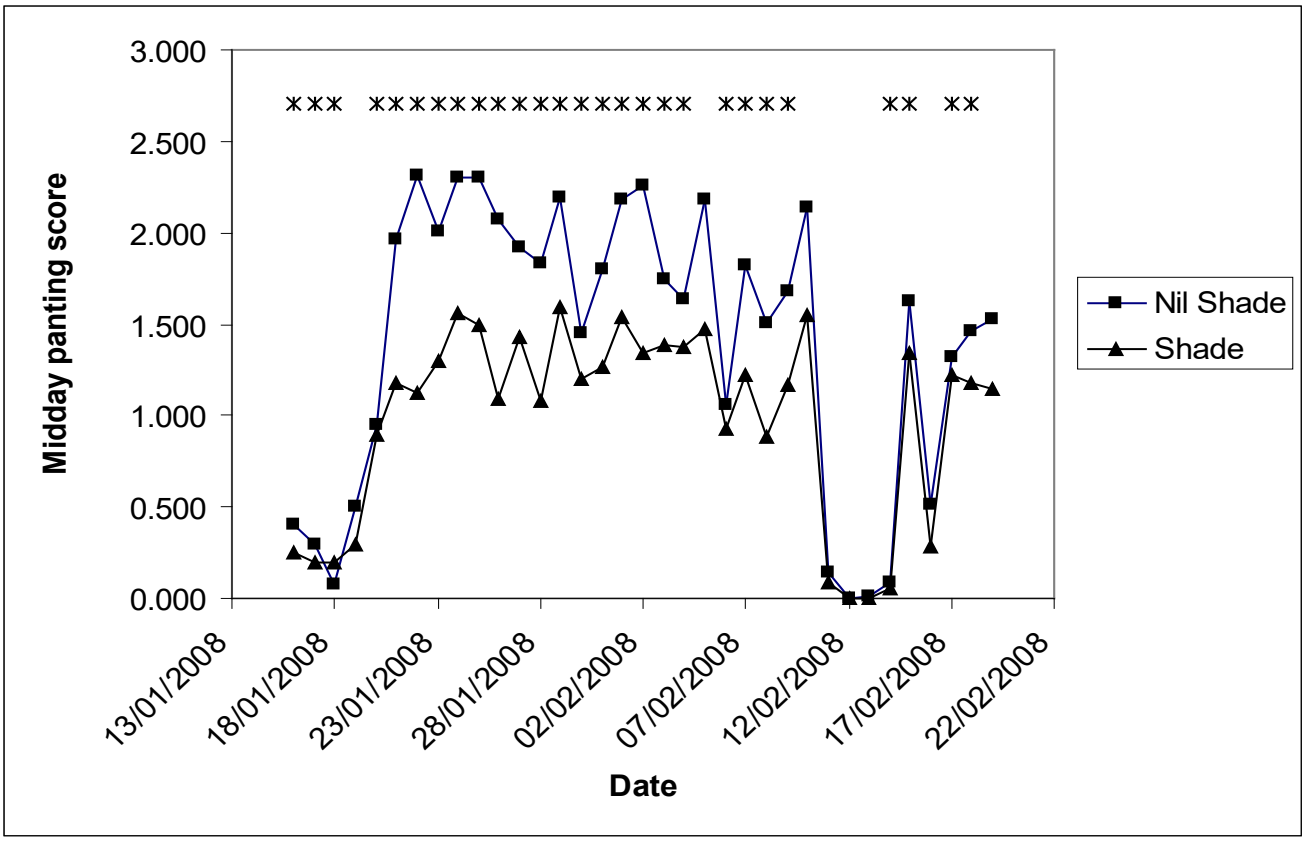

Figure 19 Effect of shade on the midday panting scores of steers over a 34 day period which includes defined hot periods. (* indicates days where dietary treatments were different (P<0.05))

The diet × shade interaction on midday panting score was significant (P<0.001) on 16 February, 2008 resulting from the elevated Glycerol-Nil Shade and Control-Nil Shade scores (Table 37).

Table 37 Diet × shade interaction on midday panting score of steers on 16 February, 2008

	16/02/2008
Control-Nil Shade	0.500^{b}
Control-Shade	0.250^{c}
Betaine 10-Nil Shade	0.313^{c}
Betaine 10-Shade	0.375^{bc}
Betaine 20-Nil Shade	0.375^{bc}
Betaine 20-Shade	0.250^{c}
Betaine 40-Nil Shade	0.375^{bc}
Betaine 40-Shade	0.313^{c}
Glycerol-Nil Shade	1.000^{a}
Glycerol-Shade	0.222^{c}
SE	0.059

Means within the column within an effect with different superscripts are significantly different (*P < 0.05*).
SE = standard error

4.16 Steer body temperature during livestock transport to abattoir

The microclimate on the respective compartments of both the top and bottom decks of the livestock transport (Truck 3) recorded with Hobo climate sensors is shown in Figure 20. The Hobo sensors were installed on Truck 3 from 0900 h onwards at feedlot exit on March 11, 2008 and the truck was loaded with temperature transmitter implanted steers between 1005 h and 1018 h. The sensors indicate an initial peak in ambient temperatures in the compartments while the truck was stationary following their installation and commencement of transit at 1025 h.

During transit, differences in temperatures between decks were small from commencement until the first stop at 1321 h were steer welfare was visually checked. Checks on steer welfare were made at subsequent stops at 1420 h and 1518 h (Logbook check). At each stop of approximately 10 minutes, temperatures on the top deck increased significantly (Sensors T1, T5 and T7) due to a lack of air movement and the incidence of solar radiation. Following the stop at 1518 h, the T1 sensor indicated temperatures remained elevated on the top deck, front compartment for an extended duration prior to declining. During the stops, the temperatures on the bottom deck remained stable. While in transit, and as ambient temperatures increased during the day, sensor temperatures increased approximately 3 °C, yet differences in sensor temperatures remained reasonably constant between decks while the truck was in motion. This reflected cooling due to wind movement.

The sensors recorded relative humidity from sensor installation to the end of the transit period at Oakey Abattoir, Oakey, Queensland (Figure 21). Sensor relative humidity tends to contrast to sensor temperature with lower relative humidity recorded on the top deck and higher on the bottom deck. While the truck was in motion, differences in relative humidity between decks were minor, varying by as little as 3 percentage units. Overall, ambient relative humidity declined during the day as ambient temperature increased. The relative humidity did not change significantly during the steer welfare stops. However, during the 1518 h (Logbook check) stop, the RH8 sensor (front right hand side of bottom deck, middle compartment) relative humidity increased significantly. In addition, from that stop onward until Oakey, the RH1 sensor (rear left hand side of top deck, front compartment) relative humidity declined and remained lower until arrival at Oakey.

The data of Figures 20 and 21 suggest that while in motion, the microclimate within all compartments of the livestock transport was relatively stable and did not compromise steer welfare.

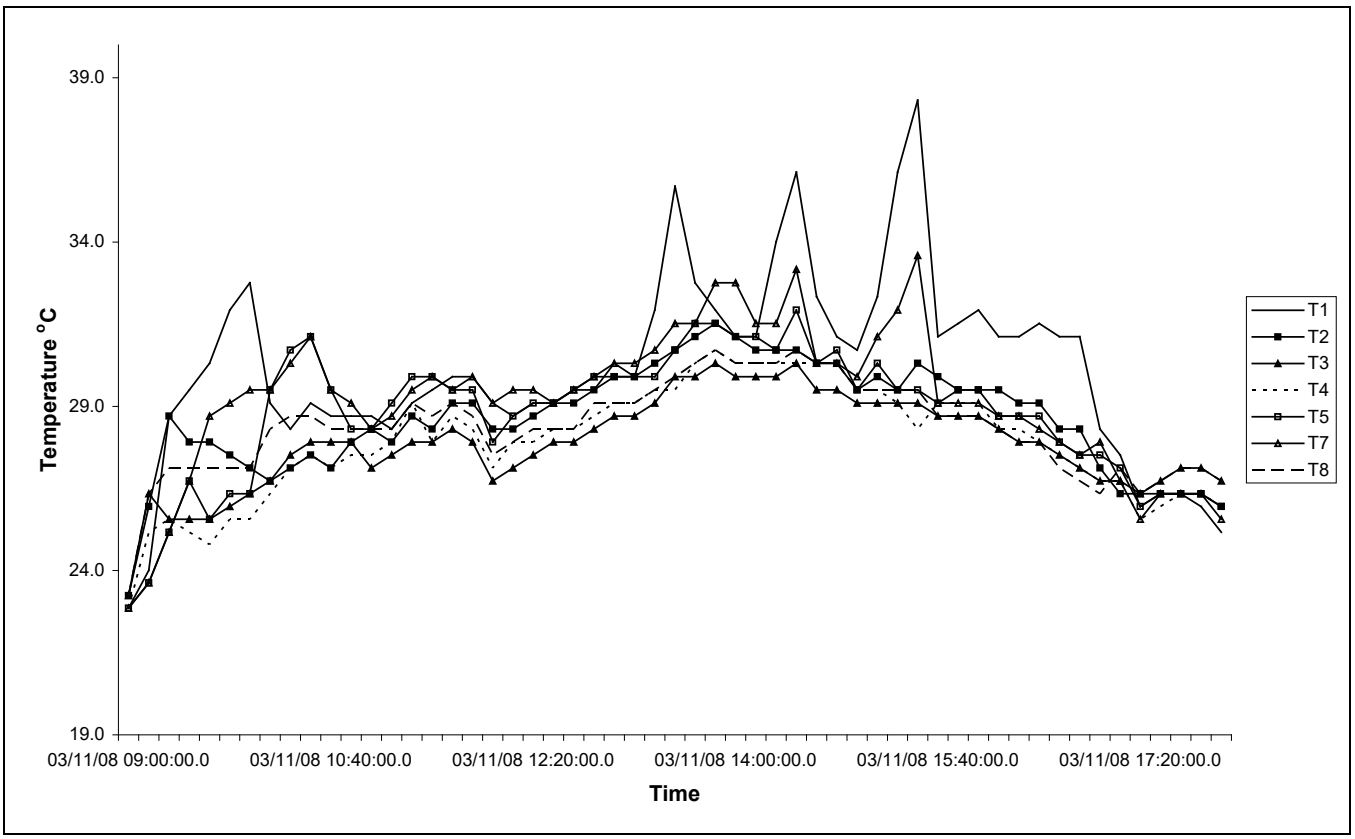

Figure 20 Ambient temperature (°C) recorded in various compartments of the Truck 3 Livestock Transport during transit between BRS and Oakey Abattoir, Oakey, Queensland. (Logger positions, T1 = rear left hand side of top deck, front compartment; T2 = towards rear left hand side of bottom deck, middle compartment; T3 = rear left hand side of bottom deck, front compartment; T4 = front right hand side of bottom deck, front compartment; T5 = front right hand side of top deck, front compartment; T7 = middle of top deck, rear compartment; T8 = front right hand side of bottom deck, middle compartment)

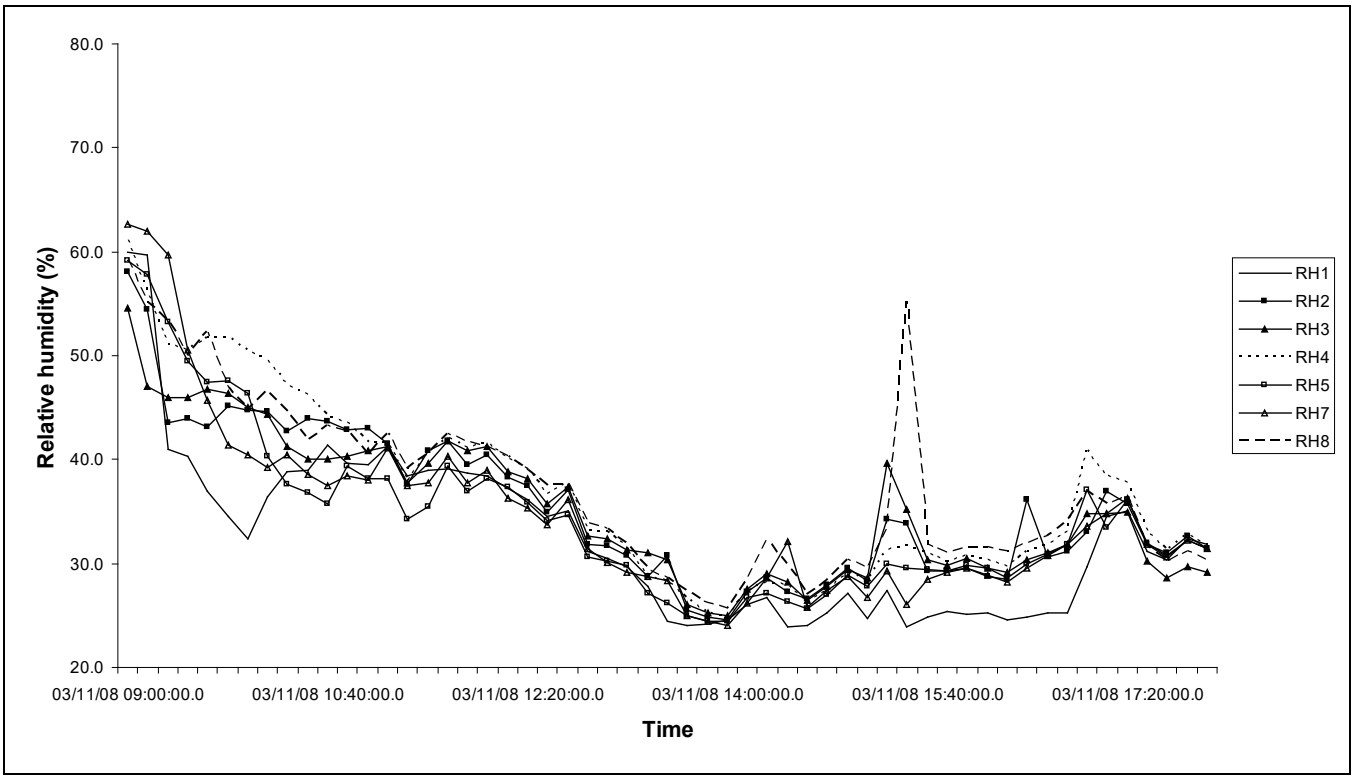

Figure 21 Relative humidity (%) recorded in various compartments of the Truck 3 Livestock Transport during transit between BRS and Oakey Abattoir, Oakey, Queensland. (Logger positions, RH1 = rear left hand side of top deck, front compartment; RH2 = towards rear left hand side of bottom deck, middle compartment; RH3 = rear left hand side of bottom deck, front compartment; RH4 = front right hand side of bottom deck, front compartment; RH5 = front right hand side of top deck, front compartment; RH7 = middle of top deck, rear compartment; RH8 = front right hand side of bottom deck, middle compartment)

Shortly after commencement of transit of the Truck 3 Livestock Transport to the abattoir, the Glycerol treatment steers recorded a higher body temperature at 1030 h (41.30 °C, $P<0.05$) than all other treatments – Control (40.84 °C), Betaine 10 (40.66 °C), Betaine 20 (40.82 °C) except Betaine 40 (41.01 °C).SE = 0.13. For the rest of the transit period dietary treatment had no influence on body temperature ($P>0.05$).

Steers from the feedlot Shade treatment recorded lower body temperatures (38.78 °C, $P<0.05$) at 1630 h prior to arrival at the abattoir at Oakey, Queensland than the steers from the Nil feedlot Shade treatment (40.10 °C; SE = 0.09). There was no diet × shade interaction.

During livestock transit, steers on the top deck of Truck 3 recorded higher mean body temperatures (°C) ($P<0.05$) than the steers on the bottom deck at 1130, 1200, 1230, 1300, 1430, 1500 and 1530 h (Figure 22). These results reflect the slightly higher microclimate temperatures on the top deck of Truck 3 (Figure 20) during the transit period while the higher body temperatures at 1430 h and 1530 h were a direct consequence of stops by the truck to check steer welfare. Figure 20 indicates the significant increase in microclimate temperatures on the top deck at these times (1430 h and 1530 h).

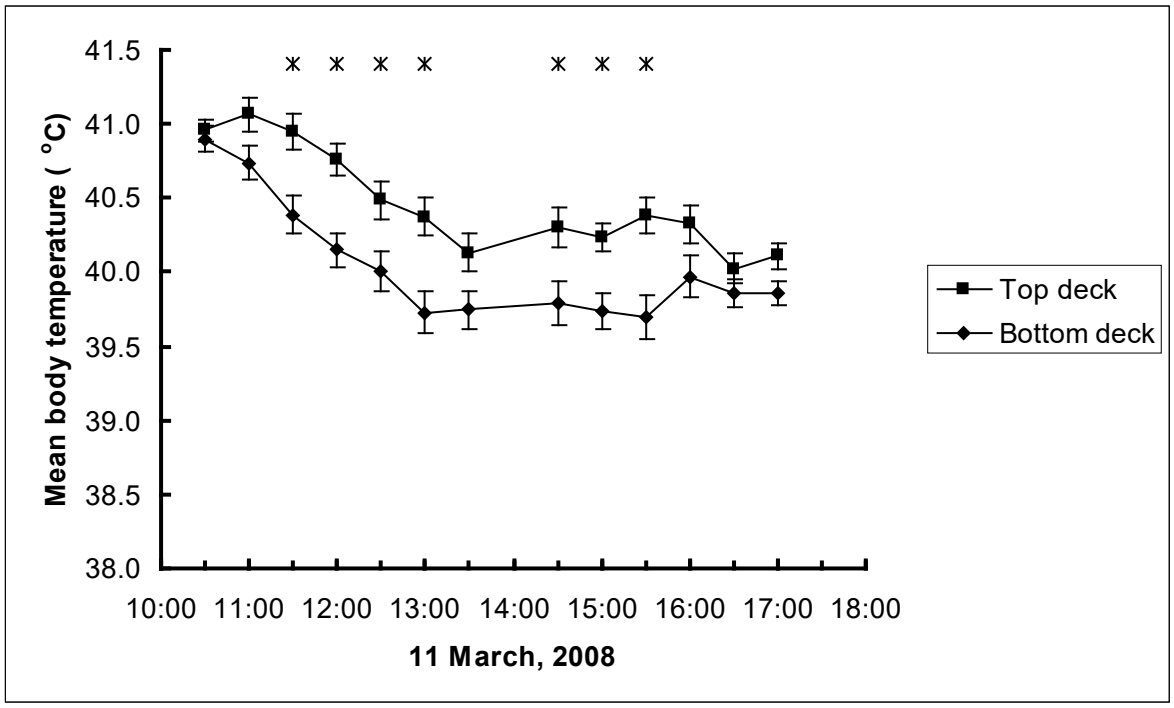

Figure 22 Mean body temperature (± SE) of steers on the top and bottom decks of the Truck 3 Livestock Transport during transit from Brigalow Research Station to Oakey Abattoir, Oakey, Queensland

4.17 Steer body temperature during abattoir lairage period

The steers were in lairage from 1800 h on 11 March to 0900 h on 12 March, 2008 (Refer to Appendix 10). Climatic conditions were described as clear and cool with a minimum overnight temperature of 15.6 °C recorded at the nearby Oakey Army Base. Body temperatures of the steers with temperature transmitters were recorded every 30 minutes during lairage. Dietary treatment had no influence (P>0.05) on body temperature of steers during lairage. Steers from the feedlot shade treatment recorded higher body temperatures (P<0.05) at 0530, 0700 and 0830 h on 12 March 2008. There was no difference due to feedlot shade during the remainder of the lairage period (Table 38). There was no diet × shade interaction.

It is unknown why the steers from the feedlot shade treatments had increased body temperatures at these random times during lairage. The only common factor is that the increase was in the morning when normal abattoir operations were proceeding. For some unknown reason, the steers from the feedlot shade treatment reacted to these operational procedures in such a way their body temperatures increased.

Table 38 Effect of feedlot shade (no shade and shade) on steer body temperature (°C) at 0530, 0700 and 0830 h during abattoir lairage

	Time		
	0530 h	0700 h	0830 h
Nil Shade	39.07[b]	38.89[b]	39.28[b]
Shade	39.23[a]	39.09[a]	39.51[a]
SE	0.04	0.06	0.05

Means within a column within an effect with different superscripts are significantly different (*P < 0.05*). SE = standard error

The mean body temperature (± SE) of steers during lairage on 11 – 12 March, 2008 is shown in Figure 23. Mean body temperature declined by over 1 °C from 40.26 °C at the commencement of lairage at 1800 h on 11 March, 2008 to a mean minimum of 38.87 °C at 0330 h on March 12, 2008. Mean body temperatures commenced to rise again from that time onwards as daily abattoir lairage operations commenced i.e. human and animal movements and pre-slaughter hygiene washings were commenced. Body temperatures of the steers rose accordingly as the steers responded to such activities and as ambient temperatures increased during the early morning.

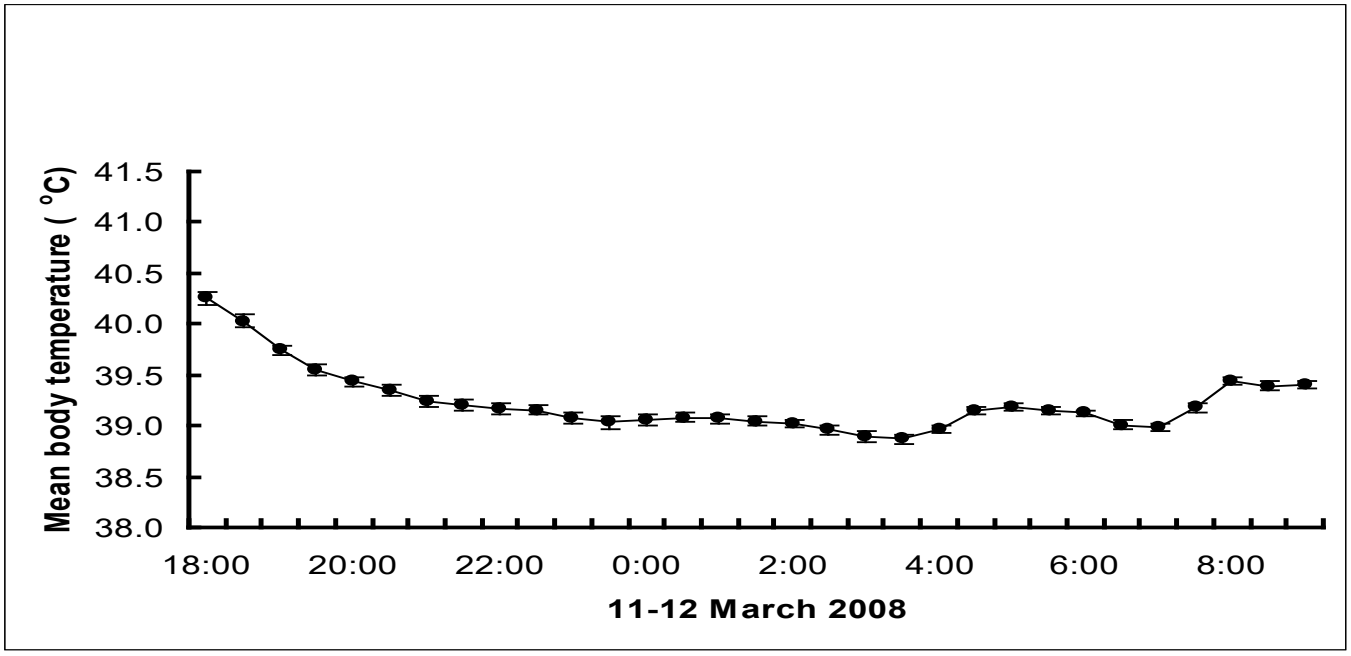

Figure 23 Mean body temperature (± SE) of steers during abattoir lairage on March 11-12, 2008

4.18 Carcase characteristics

The analysis of the abattoir data was complicated by the slaughter floor chain breakdown that occurred. As a consequence the position on the processing line was included as a linear covariate. Thus the statistical model allows for an approximately linear relationship between the variable being analysed and the processing order.

4.18.1 Slaughter data

The steers were considered young based on their dentition with 98% of the steers having zero permanent incisors at slaughter. Dentition is considered an indicator of chronological age. Bruising was insignificant being observed on one left hand carcase side only.

Apart from dressing percentage, none of the remaining slaughter data parameters were influenced ($P>0.05$) by dietary treatment (Table 39). The dressing percentage of the Glycerol treatment steers was higher ($P<0.05$) than all other treatments. This was a consequence of the numerically heavier hot standard carcase weight (HSCW) and numerically lighter exit liveweight (Table 14) of this treatment. Note that based on the study design, the glycerol effect was due to either glycerol *per se* or the pen position. As a consequence of the numerically heavier HSCW, the Glycerol treatment had a numerically

higher carcase value. The increased numerical value of the Glycerol treatment was a $24 increase over the Control.

The provision of feedlot shade resulted in heavier left side and right side weights, heavier HSCW, lower dressing percentage, higher price ($/kg HSCW) and higher carcase value (all P<0.05). The difference between exit liveweight and HSCW (an estimate of transit tissue loss) was greater (P<0.001) in the Shade treatment. There were no diet × shade interactions.

The covariate of slaughter sequence was significant for left and right side weights, HSCW and value (all P<0.01).

The benefit to the provision of feedlot shade was 6.0 kg in HSCW (1.9% over Nil Shade) and $28.44 in value of the carcase. The improvement, particularly in the value of the carcase is noteworthy.

In respect to market suitability, numerically, 25.0 % of Control, 12.5% of Betaine 10, 9.4% of Betaine 20, 21.9% of Betaine 40 and 11.1% of Glycerol treatments respectively had carcases less than 300 kg HSCW. A minimum of 300 kg HSCW is one of the key indicators of carcase suitability to the short-fed market. For shade treatments, 18.3% of Nil Shade and 13.4% of Shade treatments had carcases less than 300 kg HSCW. The differences between dietary treatments in this respect are greater than the differences between shade treatments.

Table 39 Effect of diet (Betaine 10 (10 g/steer/d), 20 (20 g/steer/d) or 40 (40 g/steer/d)) or glycerol (5% DMB) and shade (no shade and shade) and their interaction on steer carcase attributes – slaughter data

	Left side hot weight (kg)	Right side hot weight (kg)	HSCW (kg)	Dressing percentage (%)	Price ($/kg HSCW)	Value ($/carcase)	Diff. Exit liveweight to HSCW (kg)
Diet (D)							
Control	157.5	158.9	316.4	54.0[b]	3.59	1136.97	269.0
Betaine 10	159.3	160.6	319.8	54.1[b]	3.60	1151.00	271.0
Betaine 20	158.6	160.0	318.6	53.9[b]	3.59	1145.98	272.4
Betaine 40	157.4	157.8	315.1	53.8[b]	3.57	1127.44	271.2
Glycerol	160.4	161.6	321.9	55.3[a]	3.60	1160.98	260.8
SE	1.2	1.2	2.4	0.3	0.01	11.21	2.6
Shade (S)							
Nil Shade	157.1[b]	158.3[b]	315.4[b]	54.5[a]	3.58[b]	1130.25[b]	263.1[b]
Shade	160.2[a]	161.2[a]	321.4[a]	53.9[b]	3.60[a]	1158.69[a]	274.7[a]
SE	0.8	0.8	1.5	0.2	0.01	7.03	1.7
D x S							
Control-Nil Shade	156.7	158.8	315.5	54.3	3.59	1133.29	265.5
Control-Shade	158.3	159.1	317.4	53.8	3.59	1140.65	272.4
Betaine 10-Nil Shade	156.3	157.9	314.3	54.3	3.57	1124.04	264.5
Betaine 10-Shade	162.2	163.2	325.4	54.0	3.62	1177.96	277.5
Betaine 20-Nil Shade	156.1	157.0	313.1	54.1	3.57	1119.06	265.9
Betaine 20-Shade	161.2	163.0	324.2	53.7	3.61	1172.89	279.0
Betaine 40-Nil Shade	157.4	158.5	316.0	54.5	3.58	1132.07	263.3

Betaine 40-Shade	157.3	157.0	314.3	53.0	3.57	1122.82	279.0
Glycerol-Nil Shade	158.8	159.4	318.2	55.5	3.59	1142.82	256.2
Glycerol-Shade	161.9	163.7	325.6	55.1	3.62	1179.15	265.5
SE	1.7	1.8	3.4	0.4	0.01	15.81	3.7

Means within a column within an effect with different superscripts are significantly different (*P < 0.05*). SE = standard error

Neither dietary treatment (P<0.05) or the provision of shade (P<0.05) had any influence on the parameters of steer carcase fatness (Table 40). There were no diet × shade interactions.

There was no consistent numerical trend in fatness parameters to any dietary treatment or shade treatment. In respect to subcutaneous fat depth, all treatments met market specifications. Marbling overall was considered marginally moderate for both MSA-AUS·MEAT marbling score and MSA-US marbling score and reflected the relative physiological immaturity of the steers at slaughter.

The covariate of slaughter sequence was not significant for any of these variables.

Table 40 Effect of diet (Betaine 10 (10 g/steer/d), 20 (20 g/steer/d) or 40 (40 g/steer/d)) or glycerol (5% DMB) and shade (no shade and shade) and their interaction on steer carcase fatness

	P8 fat depth (hot) (mm)	Rib fat depth (cold) (mm)	MSA-AUS·MEAT marbling score	MSA-US marbling score
Diet (D)				
Control	14.9	10.9	2.59	498.9
Betaine 10	16.1	11.4	2.57	494.1
Betaine 20	15.3	10.1	2.44	489.0
Betaine 40	14.5	12.1	2.49	479.8
Glycerol	14.4	10.6	2.05	449.0
SE	0.6	0.5	0.23	23.9
Shade (S)				
Nil Shade	14.5	10.5	2.38	471.1
Shade	15.5	11.5	2.48	493.2
SE	0.4	0.3	0.15	15.0
D x S				
Control-Nil Shade	14.15	10.29	2.41	479.7
Control-Shade	15.63	11.58	2.77	518.1
Betaine 10-Nil Shade	15.52	10.86	2.46	473.7
Betaine 10-Shade	16.59	11.95	2.69	514.5
Betaine 20-Nil Shade	15.00	10.02	2.30	464.5
Betaine 20-Shade	15.59	10.17	2.59	513.6
Betaine 40-Nil Shade	13.93	11.22	2.68	490.8
Betaine 40-Shade	15.06	12.88	2.29	468.7
Glycerol-Nil Shade	14.00	10.32	2.05	446.9
Glycerol-Shade	14.80	10.93	2.05	451.0
SE	0.9	0.7	0.33	33.8

Means within a column within an effect with different superscripts are significantly different (*P < 0.05*). SE = standard error

4.18.2 Chiller assessment data

Dietary treatment had no influence (P>0.05) on cold carcase side weights, carcase shrinkage, eye muscle area or the subjective hard meat score (Table 41). Numerically there was little difference between the dietary treatments for these parameters, except for hard meat score where the Glycerol treatment had numerically less hard meat. Hard meat results in extra effort required for the manual de-boning of carcases in the Boning Room of an abattoir – a health and welfare issue for abattoir staff.

As for hot left and right side weights (Table 39), the provision of shade increased (P<0.05) both left and cold side weights. There were no differences (P<0.05) in right side shrinkage, eye muscle area and hard meat score between shade treatments. There were no diet × shade interactions.

The covariate of slaughter sequence was significant for left and right cold side weights, and eye muscle area (all P<0.01). It is not fully understood why eye muscle area was affected by position on the slaughter floor position at breakdown other than tissue hydration status may partially explain the result.

Table 41 Effect of diet (Betaine 10 (10 g/steer/d), 20 (20 g/steer/d) or 40 (40 g/steer/d)) or glycerol (5% DMB) and shade (no shade and shade) and their interaction on steer carcase attributes – chiller data

	Left side cold weight (kg)	Right side cold weight (kg)	Right side shrinkage (%)	Eye muscle area (cm^2)	Hard meat score[1]
Diet (D)					
Control	152.9	155.9	1.90	70.3	0.73
Betaine 10	154.6	157.6	1.83	71.4	0.63
Betaine 20	154.0	156.9	1.90	70.3	0.71
Betaine 40	152.8	155.0	1.77	71.3	0.84
Glycerol	155.8	158.5	1.88	70.4	0.55
SE	1.2	1.2	0.06	0.4	0.08
Shade (S)					
Nil Shade	152.4[a]	155.3[a]	1.882	70.7	0.71
Shade	155.6[b]	158.3[b]	1.830	70.8	0.68
SE	0.7	0.8	0.037	0.3	0.05
D x S					
Control-Nil Shade	152.0	155.7	1.92	70.5	0.79
Control-Shade	153.7	156.1	1.89	70.0	0.67
Betaine 10-Nil Shade	151.6	154.9	1.90	71.5	0.58
Betaine 10-Shade	157.5	160.4	1.76	71.3	0.68
Betaine 20-Nil Shade	151.6	154.0	1.92	70.2	0.64
Betaine 20-Shade	156.5	159.9	1.88	70.4	0.77
Betaine 40-Nil Shade	152.7	155.8	1.76	71.1	0.94
Betaine 40-Shade	152.8	154.2	1.79	71.5	0.75
Glycerol-Nil Shade	154.3	156.3	1.91	69.9	0.61
Glycerol-Shade	157.2	160.7	1.84	70.9	0.50
SE	1.7	1.7	0.08	0.6	0.11

[1]Hard meat score – tactile subjective rating with 0 = not hard, 1 = hard meat. Means within a column within an effect with different superscripts are significantly different (*P < 0.05*). SE = standard error

Neither dietary treatment (P>0.05) or the provision of shade influenced (P>0.05) ossification score, subjective meat colour score, pH at 18 hours post slaughter (pH_{18}), intermuscular fat , meat texture score and meat firmness score (Table 42). There were no diet × shade interactions. Ossification is an indicator of physiological age or maturity of a carcase over a range of 100 to 400. The numerical magnitude of the ossification scores indicates the relative immaturity of the steer carcase in this study. The subjective meat colour score data suggests meat of a desirable bright cherry colour, of white fat, with a low pH indicating a potential good eating quality. Both meat texture score and meat firmness score data which are both subjective tactile assessments indicate intermediate values.

The covariate of slaughter sequence was significant for subjective meat colour score (P<0.05) and meat texture score (P<0.01). The effect of the position on the slaughter floor chain at breakdown is explicable for meat colour as post mortem ageing processes have already commenced for bodies slaughtered prior to the breakdown compared to live animals processed after the breakdown. The effect of the position on the chain on meat texture score is not understood.

Table 42 Effect of diet (Betaine 10 (10 g/steer/d), 20 (20 g/steer/d) or 40 (40 g/steer/d)) or glycerol (5% DMB) and shade (no shade and shade) and their interaction on steer carcase attributes – MSA Chiller assessment of meat quality

	Ossification score	Meat[1] colour	pH_{18}[2]	Intermuscular fat colour	Meat[3] texture score	Meat[4] firmness score
Diet (D)						
Control	123.6	0.27	5.44	0.75	2.99	2.75
Betaine 10	124.1	0.24	5.46	0.81	2.88	2.69
Betaine 20	122.3	0.32	5.45	0.79	2.95	2.76
Betaine 40	121.4	0.35	5.45	0.90	2.74	2.47
Glycerol	123.5	0.26	5.46	0.96	2.73	2.43
SE	2.0	0.08	0.01	0.06	0.10	0.12
Shade (S)						
Nil Shade	123.5	0.35	5.45	0.89	2.83	2.60
Shade	122.4	0.22	5.45	0.80	2.88	2.64
SE	1.3	0.05	0.00	0.04	0.06	0.07
D x S						
Control-Nil Shade	125.4	0.39	5.45	0.81	3.05	2.72
Control-Shade	121.9	0.16	5.44	0.69	2.93	2.78
Betaine 10-Nil Shade	125.0	0.29	5.45	0.81	2.69	2.62
Betaine 10-Shade	123.1	0.18	5.46	0.82	3.07	2.75
Betaine 20-Nil Shade	122.2	0.46	5.45	0.88	2.81	2.49
Betaine 20-Shade	122.4	0.18	5.45	0.70	3.09	3.02
Betaine 40-Nil Shade	121.3	0.38	5.44	0.94	2.75	2.56
Betaine 40-Shade	121.5	0.32	5.45	0.87	2.74	2.38
Glycerol-Nil Shade	123.7	0.25	5.46	1.01	2.86	2.60
Glycerol-Shade	123.3	0.27	5.46	0.92	2.59	2.26
SE	2.9	0.11	0.01	0.09	0.15	0.16

[1]Meat colour was coded as either 1B (n=117), 1C (n=43) or 2 (n=4), then converted to a single integer variable - 1B=0 and >1C=1
[2]pH_{18} = pH measured at 18 hours post slaughter
[3]Texture score, 1 = coarse, 5 = fine
[4]Firmness score, 1 = soft, 5 = firm

Means within a column within an effect with different superscripts are significantly different (*P < 0.05*). SE = standard error

Neither diet (P>0.05) nor the provision of feedlot shade (P>0.05) had any influence on the objective carcase chiller measurement of surface meat colour, drip loss and the oxymyoglobin-metmyoglobin (OM) ratio of the LD at the carcase quartering point (Table 43). There were no diet*shade interactions.

The HunterLab Miniscan 'L' (indicating surface meat lightness), 'A' (indicating surface meat redness) and 'B' (indicating surface meat yellowness) were considered to be normal for colour measurements at the completion of the carcase chilling cycle. The drip loss values suggest significant weep/wetness was recorded on the freshly cut meat surface at the quartering point. Weep or drip is associated with product storage loss and should be minimal. OM ratios were considered sufficient to ensure significant shelf life of the resultant meat. The OM ratio is designed to indicate potential meat display life and is reported to measure the oxymyoglobin predominance over metmyoglobin in the surface (Warner *et al.* 2007). Changes in the myoglobin content of the meat or in the glycolytic-oxidative mechanism can alter the oxidative potential of the meat post-slaughter and thus the formation of metmyoglobin in the meat and the meat's subsequent shelf-life. In ovines, the reference ratio used is 3.5 to compare the rates in change in OM between factors such as dietary treatment or genotypes (Warner *et al.* 2007).

The covariate of slaughter sequence was significant for Minolta surface colour scores 'L' (P<0.05) and 'B' (P<0.01). As for subjective meat colour score (Table 42), the effect of the position on the slaughter floor chain at breakdown may also explain the effect of position on objective meat colour as post mortem ageing processes have already commenced for bodies slaughtered prior to the breakdown compared to live animals processed after the breakdown.

Table 43 Effect of diet (Betaine 10 (10 g/steer/d), 20 (20 g/steer/d) or 40 (40 g/steer/d)) or glycerol (5% DMB) and shade (no shade and shade) and their interaction on the objective carcase chiller measurement of surface meat colour, drip loss and the oxymyoglobin-metmyoglobin (OM) ratio of the LD at the carcase quartering point

	HunterLab Miniscan colour scores				
	L	A	B	Drip loss (%)	OM Ratio
Diet (D)					
Control	33.4	27.5	25.2	50.4	10.8
Betaine 10	34.0	27.4	25.4	54.2	10.8
Betaine 20	33.7	26.8	24.8	52.0	10.5
Betaine 40	34.7	27.1	25.6	54.2	10.4
Glycerol	33.6	27.7	25.6	53.8	11.1
SE	0.5	0.6	0.5	5.1	0.5
Shade (S)					
Nil Shade	33.7	27.2	25.2	53.1	10.7
Shade	34.1	27.4	25.4	52.8	10.8
SE	0.3	0.4	0.3	3.2	0.3
D x S					
Control-Nil Shade	33.1	27.9	25.4	54.8	11.1
Control-Shade	33.8	27.0	25.0	46.0	10.5
Betaine 10-Nil Shade	34.0	27.0	25.2	55.4	10.7
Betaine 10-Shade	33.9	27.8	25.5	53.1	11.0
Betaine 20-Nil Shade	33.4	26.7	24.7	50.6	10.4
Betaine 20-Shade	34.0	26.9	24.9	53.5	10.7
Betaine 40-Nil Shade	34.1	26.8	25.1	52.9	10.5
Betaine 40-Shade	35.4	27.5	26.1	55.6	10.4
Glycerol-Nil Shade	33.7	27.6	25.5	51.8	10.9
Glycerol-Shade	33.5	27.9	25.7	55.8	11.3
SE	0.7	0.8	0.7	7.2	0.7

Means within a column within an effect with different superscripts are significantly different ($P < 0.05$). SE = standard error

4.19 Meat quality

The loin temperature-pH decline measured over a three hour period post slaughter indicated that all carcases regardless of treatment or position on the slaughter floor chain at breakdown were heat shortened i.e. they reached a pH of 6.0 at a greater temperature than the preferred 20 °C (Anon 2003).

Neither dietary treatment (P>0.05) or the provision of shade influenced (P>0.05) ultimate pH (pH_u) or objectively measured meat colour (Table 44) of LD steak samples. There were no diet × shade interactions.

The ultimate pH values were ideal and towards the lower end of the normal range. The Minolta 'L' colour score values indicate meat lightness and samples were considered to be of optimum lightness. The Minolta 'A' colour score values indicate meat 'redness' and the samples were considered to be of a desirable optimum cherry red colour The optimum values of ultimate pH and meat colour indicate an absence of pre slaughter stress and any resultant dark cutting.

The covariate of slaughter sequence was significant for Minolta colour 'A' (P<0.001). As for subjective meat colour score (Table 42) and objectively measured surface meat colour in the chiller (Table 43), the effect of the position on the slaughter floor chain at breakdown may also explain the effect of the position on chain on objective meat colour of LD steaks as post mortem ageing processes have already commenced for bodies slaughtered prior to the breakdown compared to live animals processed after the breakdown.

Table 44 Effect of diet (Betaine 10 (10 g/steer/d), 20 (20 g/steer/d) or 40 (40 g/steer/d)) or glycerol (5% DMB) and shade (no shade and shade) and their interaction on the ultimate pH (pH_u) and meat colour of LD steak samples as assessed by Minolta colour scores

	pH_u	Minolta colour scores	
		L	A
Diet (D)			
Control	5.45	38.9	20.2
Betaine 10	5.45	39.2	20.0
Betaine 20	5.45	38.8	19.8
Betaine 40	5.44	39.8	19.6
Glycerol	5.45	38.7	20.3
SE	0.01	0.4	0.3
Shade (S)			
Nil Shade	5.45	38.7	19.7
Shade	5.45	39.5	20.2
SE	0.00	0.3	0.2
D × S			
Control-Nil Shade	5.45	38.1	20.1
Control-Shade	5.45	39.6	20.3
Betaine 10-Nil Shade	5.45	38.7	20.0
Betaine 10-Shade	5.45	39.7	20.0
Betaine 20-Nil Shade	5.46	38.4	19.4
Betaine 20-Shade	5.45	39.2	20.2
Betaine 40-Nil Shade	5.45	39.7	19.2
Betaine 40-Shade	5.43	39.9	20.0
Glycerol-Nil Shade	5.45	38.5	20.0
Glycerol-Shade	5.46	39.0	20.6
SE	0.01	0.6	0.4

Means within a column within an effect with different superscripts are significantly different (*P < 0.05*). SE = standard error

Dietary treatment had no influence (P<0.05) on the total fat content, moisture or calculated chemical lean content of LD steak samples (Table 45). The provision of shade increased total fat content (P<0.05), reduced moisture (P<0.05) and reduced the calculated chemical lean content (P<0.05) of LD steak samples. There were no diet × shade interactions.

The total fat content of the LD samples was high for animals considering their physiological immaturity and when compared against the marginally moderate marbling scores of Table 40. While the total fat content was considered high for animals considered relatively immature, meat trimmings from similar primal cuts would have been packed for export markets in 90 CL 'red meat packs', which is considered a relatively lean product.

The covariate of slaughter sequence was not significant (P>0.05) for LD steak fat content, moisture and calculated chemical lean content.

Table 45 Effect of diet (Betaine 10 (10 g/steer/d), 20 (20 g/steer/d) or 40 (40 g/steer/d)) or glycerol (5% DMB) and shade (no shade and shade) and their interaction on LD steak fat content, moisture and calculated chemical lean content (CL)

	Total fat (%)	Moisture (%)	Calculated CL (%)
Diet (D)			
Control	7.89	70.7	92.1
Betaine 10	7.71	70.9	92.3
Betaine 20	7.37	71.2	92.4
Betaine 40	7.60	71.0	92.4
Glycerol	7.20	71.3	92.8
SE	0.30	0.2	0.3
Shade (S)			
Nil Shade	7.16[b]	71.3[a]	92.8[a]
Shade	7.94[a]	70.7[b]	92.1[b]
SE	0.19	0.2	0.2
D x S			
Control-Nil Shade	7.08	71.4	92.91
Control-Shade	8.71	70.1	91.29
Betaine 10-Nil Shade	7.49	71.1	92.52
Betaine 10-Shade	7.92	70.7	92.08
Betaine 20-Nil Shade	6.84	71.6	92.75
Betaine 20-Shade	7.89	70.7	92.11
Betaine 40-Nil Shade	7.53	71.0	92.48
Betaine 40-Shade	7.67	70.9	92.35
Glycerol-Nil Shade	6.87	71.6	93.10
Glycerol-Shade	7.52	71.0	92.49
SE	0.43	0.3	0.5

Means within a column within an effect with different superscripts are significantly different (*P < 0.05*). SE = standard error

The percentage distribution of fatty acids within adipose carcase fat were not (P>0.05) affected by diet or shade (Table 46). However, the effects of diet × shade were significant (P<0.05) for 18:1cis9 (oleic acid). Note that the data of this table needs to be interpreted with caution as the data has been analysed with animal level variance due to the sampling strategy.

Table 46 Effect of interaction between diet (Betaine 10 (10 g/steer/d), 20 (20 g/steer/d) or 40 (40 g/steer/d)) and shade (no shade and shade) on percentage composition of individual fatty acids within adipose fat over the *Longissimus dorsi*

	Fatty acid (%)														
	14:0	14:1	16:0	16:1 (w-7)	18:0	18:1 cis9	18:1 trans11	18:2 (n-6)	18:3 (n-6)	18:3 (n-3)	20:0	20:1	20:4 (w-6)	Total saturated	Mono/poly-un:saturated
Control															
Nil shade	3.57	0.50	28.79	2.51	18.7	43.2^{bd}	0.59	1.34	0.00	0.20	0.10	0.40	0.05	51.20	0.96
Shade	3.30	0.38	26.98	2.34	19.0	45.0^{ad}	0.73	1.69	0.02	0.08	0.08	0.34	0.02	49.40	1.03
Betaine 10															
Nil Shade	3.37	0.37	27.98	2.50	17.8	45.2^{ac}	0.95	1.40	0.02	0.08	0.03	0.27	0.00	49.20	1.03
Shade	3.70	0.49	28.98	2.57	17.6	44.0^{bcd}	0.66	1.54	0.02	0.07	0.03	0.33	0.03	50.31	0.99
Betaine 20															
Nil shade	3.46	0.44	27.79	2.30	19.2	43.8^{bcd}	0.89	1.54	0.03	0.05	0.13	0.36	0.06	50.54	0.98
Shade	3.45	0.56	27.78	2.61	17.0	46.1^{a}	0.55	1.45	0.03	0.07	0.07	0.34	0.02	48.30	1.07
Betaine 40															
Nil shade	3.40	0.37	27.43	2.25	18.6	45.5^{ac}	0.23	1.58	0.04	0.08	0.06	0.42	0.02	49.54	1.02
Shade	3.62	0.53	27.95	2.58	17.4	45.4^{ac}	0.38	1.48	0.03	0.07	0.06	0.45	0.00	49.07	1.04
SE	0.18	0.07	0.56	0.19	0.8	0.7	0.30	0.12	0.02	0.04	0.04	0.07	0.02	0.76	0.03
F-prob															
Diet × Shade	0.33	0.16	0.07	0.50	0.36	0.03	0.72	0.19	0.87	0.22	0.75	0.87	0.18	0.11	0.12

Means within a column for each month with superscripts are significantly different ($P < 0.05$).
SE = standard error, d = day

Neither diet (P>0.05) nor the provision of feedlot shade (P>0.05) had any influence on the objective determinants of meat quality (Table 47) of LD steak samples. There were no diet × shade interactions.

Sarcomere lengths were optimal and suggest little evidence of muscle shortening in this study. Of all samples, 7/164 (4.3%) had sarcomere lengths <1.8 μm, a value below which some muscle shortening might be expected to occur. Of the 7 samples with sarcomere lengths <1.8 μm, 4 of these were from steers in the first 39 steers to be slaughtered and that were already in the carcase chiller at the time of the slaughter floor chain breakdown. Overall, the magnitude of the sarcomere lengths is in contrast to the fact that all carcases were suggested to be heat shortened according to the guidelines of Anon (2003) where carcase are stated to be heat shortened if they reach a pH of 6.0 at a greater temperature than the preferred 20 °C.

Cooking losses are associated with juiciness of meat and values were considered normal for LD samples cooked at 70 °C.

The Modified Warner Bratzler values for Initial Yield (indicates the myofibrillar contribution component of meat tenderness/toughness), for Peak Force (indicator of overall tenderness) and for PF-IY (an index of connective tissue contribution to meat tenderness/toughness) were all low, indicating LD steak samples of excellent tenderness.

The PF-IY and Instron compression data both indicate the contribution of connective tissue to tenderness/toughness of meat samples. Instron Compression is of greatest benefit in indicating the contribution of connective tissue to meat tenderness/toughness when muscle shortening is minimal, which was the case in this study. Overall, the LD is a muscle where there is little contribution of connective tissue to meat tenderness/toughness, compared to other muscles. The values for Instron Compression were very low in this study. Thus due to minimal muscle shortening combined with the youth of the steers at slaughter, there was little connective tissue contribution to impact on the tenderness of the LD steak samples.

Based on the parameters of objective meat analysis, the data for LD steaks suggests the meat from the steers in this study to be of excellent quality in respect to tenderness.

The covariate of slaughter sequence was significant for sarcomere length (P<0.05) and for Instron compression (P<0.01). The result for Instron compression is not explicable, however it is understandable that position on the slaughter floor chain at breakdown may have affected chilling times/rates in the early part of the chilling process for carcase sides already in the chiller in comparison to steers not slaughtered at the time of breakdown.

Table 47 Effect of diet (Betaine 10 (10 g/steer/d), 20 (20 g/steer/d) or 40 (40 g/steer/d)) or glycerol (5% DMB) and shade (no shade and shade) and their interaction on the objective tenderness of LD steak samples

			Modified Warner Bratzler Shear Force			
	Sarcomere length (µm)	Cooking loss (%)	Initial yield (IY;kg)	Peak Force (PF;kg)	PF-IY (kg)	Instron compression (kg)
Diet (D)						
Control	1.95	20.1	3.10	3.80	0.69	1.16
Betaine 10	1.91	19.9	2.88	3.60	0.72	1.11
Betaine 20	1.91	20.0	2.92	3.68	0.76	1.12
Betaine 40	1.94	20.3	2.86	3.74	0.88	1.16
Glycerol	1.95	20.7	3.15	3.94	0.79	1.23
SE	0.02	0.3	0.12	0.11	0.07	0.04
Shade (S)						
Nil Shade	1.94	20.3	3.07	3.84	0.77	1.17
Shade	1.92	20.1	2.90	3.66	0.76	1.15
SE	0.02	0.2	0.08	0.07	0.04	0.02
D x S						
Control-Nil Shade	1.96	20.1	3.20	3.95	0.74	1.18
Control-Shade	1.93	20.1	3.00	3.65	0.64	1.14
Betaine 10-Nil Shade	1.93	20.1	2.95	3.61	0.66	1.08
Betaine 10-Shade	1.89	19.6	2.82	3.59	0.77	1.15
Betaine 20-Nil Shade	1.90	19.7	3.09	3.79	0.71	1.12
Betaine 20-Shade	1.93	20.3	2.76	3.57	0.82	1.13
Betaine 40-Nil Shade	1.93	20.3	2.91	3.71	0.80	1.21
Betaine 40-Shade	1.95	20.3	2.81	3.77	0.96	1.10
Glycerol-Nil Shade	1.97	21.3	3.20	4.14	0.94	1.25
Glycerol-Shade	1.92	20.1	3.11	3.74	0.63	1.22
SE	0.03	0.4	0.17	0.16	0.09	0.05

Means within a column within an effect with different superscripts are significantly different ($P < 0.05$). SE = standard error

4.20 Effect of surgical implantation of temperature transmitter on subsequent animal performance

Because of the importance of the procedure for surgical implantation of temperature transmitters (surgery) to the objective of the study, the direct effect of surgery on subsequent animal performance was analysed.

The direct effect of surgery was recorded for the following parameters:

- Induction liveweight (P<0.01) +8.82 kg. An expected result which is not understood.
- Change in 30d – 60d hip height (P<0.05). +8.76 mm. A consequence of low hip height gain from I – 30d.

- Steer 60d USBCS (P<0.001). +0.20 score. A consequence of lower USBCS at I and 30d.
- Steer 110d USBCS (P<0.05). +0.12 score. Cannot be explained.
- Change in 30d – 60d (P<0.05) USBCS. +0.11 score. A consequence of lower change from I – 30d.
- Change in I – 60d USBCS. +0.24 score. A cumulative change from Induction to 60d.
- Change in I – 110d USBCS. +0.17 score. Not understood.
- Left side hot weight (P<0.05). -4.56 kg. Not understood as the surgery had been carried out on the right side, not left side.
- Diff. Exit liveweight to HSCW (P<0.05). -9.94 kg. Not understood as surgery had no direct effect on either Exit liveweight or HSCW.
- Left side cold weight (P<0.05). -4.71 kg. Not understood as the surgery had been carried out on the right side, not left side.
- Right side shrinkage (P<0.05). +0.24 %. May reflect tissue stasis surrounding surgery site.
- Hard meat score (P<0.05). +0.35 score. Not understood.
- Visually assessed meat colour (P<0.05). +0.28 score. Reflects position on slaughter floor chain at breakdown.
- Meat texture score (P<0.01). -0.48 score. Not understood.
- Post chilling objective meat colour 'A' score (P<0.05). -1.70 score. Reflects position on slaughter floor chain at breakdown.
- pH_u (P<0.01). +0.04 units. Steers with transmitters responded in a slightly negative way to pre-slaughter stress.
- LD steak objective meat colour 'A' score (P<0.01). -1.62 score. Reflects position on slaughter floor chain at breakdown.

In summary the surgical procedure impacted in the short term on some live animal parameters as expected, impacted on some weight based carcase parameters which are not understood and influenced meat colour which may be explained by the position of the carcase on the slaughter floor chain at the time of the breakdown.

These results indicate the surgical procedure used in the study met the objective of implantation of temperature transmitters without compromising welfare or performance of the steers.

5 Success in achieving objectives

The climatic conditions which prevailed over the data collection period were sufficient to induce heat stress in the Angus cattle, although there were only a couple of events during which the cattle were exposed to extreme heat load. Overall the climatic conditions would have been harsh enough to elicit a response from the dietary and shade treatments. Clear welfare and performance differences were seen between shaded and un-shaded cattle. Therefore the project has been able to achieve the objectives as set out in section 2 of this report.

6 Impact on meat and livestock industry – now & in five years time

The findings from this study (B.FLT.0345) have provided for the first time a scientific basis to the use of betaine and glycerol in diets fed to finishing cattle over the summer months in Australian feedlots. This studies suggest that there is no benefit of adding betaine or glycerol to the diets of *Bos taurus* feedlot cattle as a method of heat alleviation over the summer months.

However clear positive, measurable welfare outcomes and production responses have been demonstrated in the current study (and in B.FLT.0343) when shade is used for Angus cattle over the summer months. In the current study shaded improved returns by $40.69 per head over 120 day feeding period.

The use of shade will not only improve animal welfare, and will improve public perception of the welfare of feedlot cattle. This will have both short and longer term benefits for the feedlot industry.

7 Conclusions and recommendations

7.1 Conclusions

Based on the production and welfare findings from this study, the genotype of cattle used (Black Angus), the stocking density, the diets and shade structure used in the current study the following conclusions are made.

1. Betaine inclusion in the diet did not improve performance or reduce the impact of high heat load. This finding was unexpected given the positive responses in animal house studies and the anecdotal evidence form commercial feedlots. We can only speculate that there may be dietary ingredient interactions which may reduce the efficacy of betaine.
2. Glycerol inclusion in the diet did not reduce the impact of high heat load.
3. There was a positive response of feeding glycerol in terms of HSCW, however the high cost of freight associated with glycerol in the current study (essentially doubled the cost of glycerol) resulted in a dollar return that was below the control.
4. Access to shade reduced the impact of extreme conditions but did not completely eliminate heat stress.
5. The cattle increased shade usage when the HLI>86. This suggests that the Risk Analysis Program thresholds are correct for the reference animal.
6. The relative humidity value in the HLI equation appears too high when black globe temperature is below 25^{o}C. This results in an overestimation of the impact of climatic conditions (AHLU) especially in the mornings.
7. During periods of high heat load cattle with access to shade had lower midday mean panting scores (20 to 30% lower). This indicates that the cattle with access to shade do not need to 'work' as hard to maintain body temperature via panting.
8. During periods of high heat load cattle with access to shade had less variation in mean body temperature (0.9 – 1.5^{o}C) compared to cattle without access to shade (1.5 – 2.6^{o}C).

Maximum body temperature were greater for non-shaded *cv.* shaded cattle (41.7ºC and 40.5ºC respectively). These results suggests that shaded cattle are better able to regulate body temperature because they are not exposed to the maximum solar load.

9. There was considerable individual variation in terms of body temperature and panting responses to high heat load.
10. The shaded cattle had a better feed efficiency than did the un-shaded cattle at 6.25:1 and 6.60:1 respectively. Based on a 100 kg weight gain and feed at $300/t, the cost of feeding the non-shaded cattle was $12.25 greater than the shaded cattle.
11. Shaded cattle had lower dressing percentage but overall higher HSCW (6 kg) than non-shaded cattle.
12. Based on conclusion 10, the shaded cattle returned $28.44 per head more than the non-shaded cattle. When conclusion 9 is included the shaded cattle returned $40.69 per head more than the non-shaded cattle.
13. Cattle fed glycerol had a greater dressing percentage than the other dietary treatment groups.
14. Land transport did not adversely impact on body temperature. The cattle on the top deck had a higher body temperature than those on the lower deck. This was most likely due to the effect of solar load.

7.2 Recommendations

Based on the production and welfare findings from this study, the type of cattle used, the stocking density, the dietary treatments and shade area used the following recommendations are made.

Recommendation 1: Shade should be considered as the primary method to alleviate heat load for black *Bos taurus* feedlot cattle in areas were high heat load is expected. Shade will improve animal welfare and production.

Recommendation 2: The findings from the study be disseminated to the feedlot sector before summer 2009/10.

Recommendation 3: The HLI equation will need to be modified to reflect the lesser impact of relative humidity when the black globe temperature is less than 25ºC. This should be undertaken in conjunction with Recommendation 4, prior to summer 2009/10.

Recommendation 4: Further statistical analysis should be undertaken of the data in order to further understand the physiological responses of cattle to high heat load. This should include data collected from previous heat load studies. The information obtained from this would further strengthen the heat load model.

Recommendation 5: Based on the greater HSCW of the cattle fed glycerol it is recommended that a replicated study be undertaken to further investigate the effects of feeding glycerol. (This recommendation is based on the assumption that the expansion of the ethanol industry in Queensland will result in glycerol being locally available resulting in a reduction in freight costs).

8 Acknowledgements

We thankfully acknowledge the support and assistance of the Queensland Department of Primary Industry & Fisheries staff at Brigalow Research Station, Theodore, Mr Will Cameron, Cameron Livestock for sourcing the trial cattle, David Reid, DPI&F (Qld), Rockhampton for initial allocation of the trial cattle, Dr Rod Howard, formerly Roma Veterinary Clinic, Roma Qld for carrying out the surgical procedure for implanting the temperature transmitters, Sonya Fardell, The University of Queensland Gatton Qld for assisting Dr Howard, the management and staff of Oakey Abattoir, Qld, Janine Lau and David Strachan of MLA/MSA for specific carcase measurements and Nick Kempe and David Cadogan of Feedworks for their financial support of the project.

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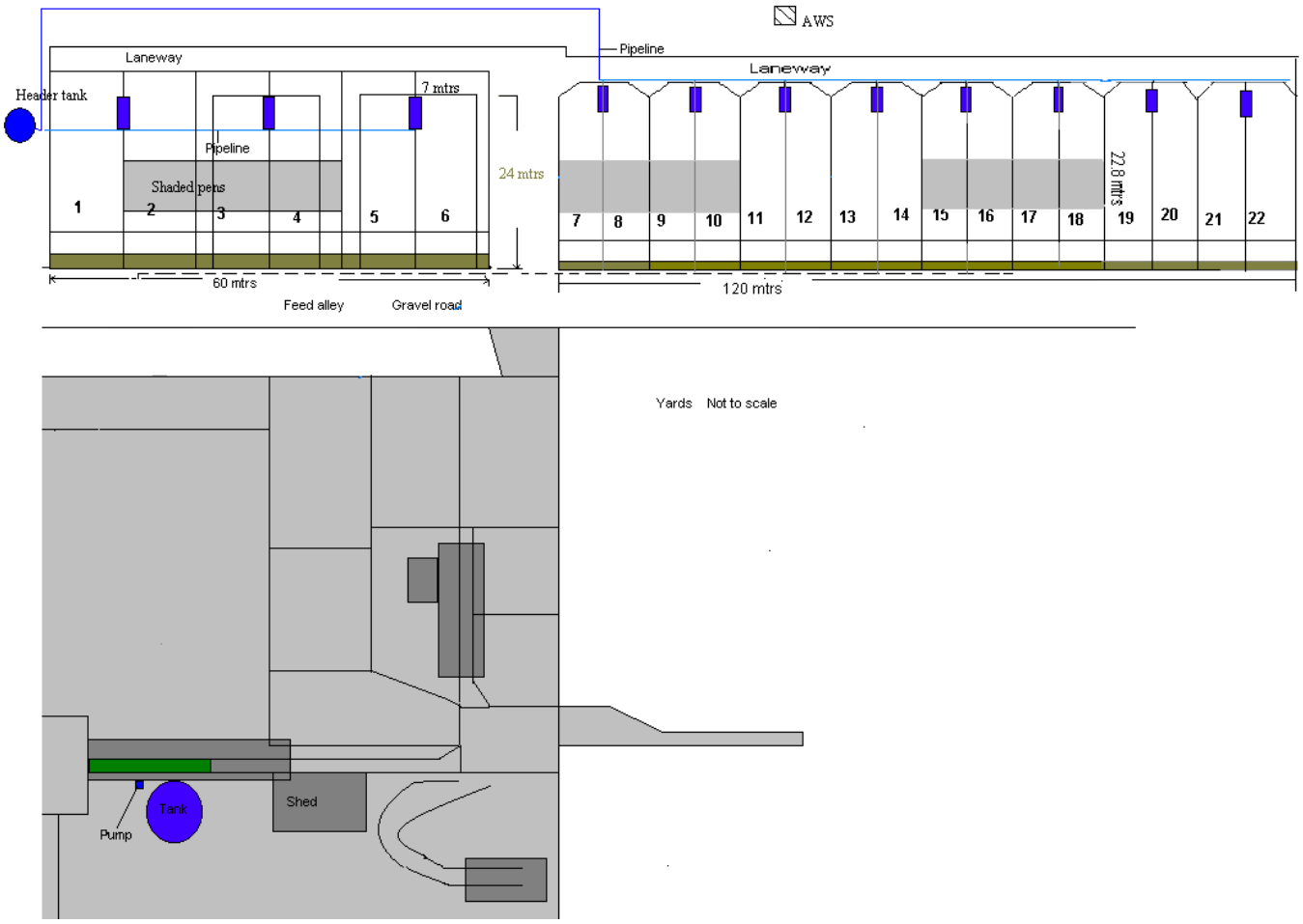
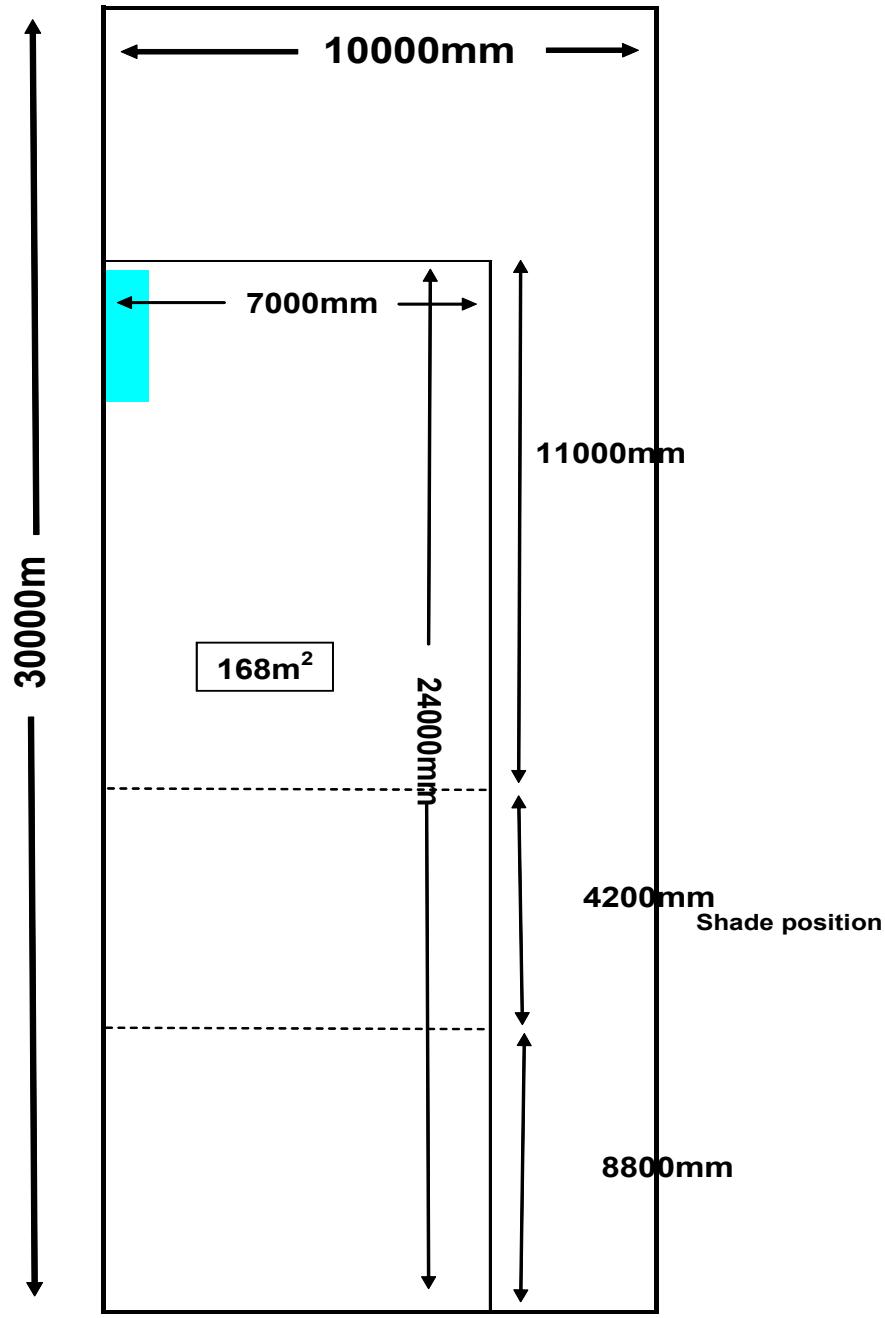
10 Appendices

10.1 Appendix 1 Feedlot layout – Brigalow Research Station



10.2 Appendix 2 Pen dimensions for pens 3 – 4



10.3 Appendix 3 MLA Betaine/Shade Project Date and Measurement Schedule - Live Animal Phase

Wednesday September 5, 2007 – Receive 177 Steers at Moura saleyards due to impassable road conditions to Brigalow
Steers placed on hay and water in yards.

Thursday September 6, 2007
Procedures and measurements carried out at Moura saleyards.
AM
- NLIS Scan
- Vaccinated with trivalent tick fever vaccine.
- 6 x animals treated for BIK infections
- Cattle retained in yards overnight with access to hay and water

Friday September 7, 2007
Two decks (i.e. 60 hd) transported from Moura saleyards to Brigalow Research Station.
Cattle given access to lucerne hay and well pastured paddock
Road conditions still poor and decision made to hold the balance of animals at Moura until conditions improved.

Sunday September 9, 2007
Balance of steers (i.e. 117 hd) transported to Brigalow on 2 x 2 decks
Cattle given access to lucerne hay and well pastured paddock.

Monday September 10, 2007
Animals walked to main yards
- 1st flight speed recorded
- Animals returned to pasture

Tuesday September 11, 2007
Animals walked to main yards
- Vaccinated with 1st dose BEF
- Vaccinated 'Longrange' Botulinum vaccine
- Applied Cydectin pour-on
- Measured liveweight
- Estimated Hip Height
- Replaced visual ID tags
- Checked all eyes, treated 10 animals for BIK infections
- Animals returned to pasture

Monday September 17, 2007
Walk animals to main yards
- Measure 2nd Flight speed
- Check eyes and treat as necessary
- Brand steers

Tuesday October 2, 2007
Walk animals to main yards

– Vaccinate with 2nd dose BEF vaccine

Sunday October 7, 2007 - Procedures and measurements carried out on individual animals in the Main Race of the cattle yards. Working cattle yards wetted and races/crushes hosed down immediately prior to this day
- Test weight of liveweight scales
- From 7:00 AM, steers walked to Main Yards from paddock.
- Steers remain off feed and water while in yards
- Measure rectal temperature using graduated thermometers
- Estimate US Body Condition Score (USBCS) using method of Herd and Sprott (1996)
- Confirm phenotype
- Estimate visual hip height
- Measure non fasted liveweight
- Walk steers to well pastured 40 ha paddock close to cattle yards
- Working cattle yards wetted and races/crushes hosed down

Monday October 8, 2008 - Procedures and measurements carried out on individual animals in the Main Race of the cattle yards
- Steers walked to Main Yards from paddock.
- Steers remain off feed and water while in yards
- Draft off 63 steers allocated for surgical implantation
- Walk 63 steers for surgical implantation to 20 ha paddock adjacent to yards
- Walk remaining 113 steers to a separate well pastured paddock
- Working cattle yards wetted and races/crushes hosed down (With a Gerni or Karcher if available).

Tuesday October 9, 2007 – Surgical implantation of temperature transmitters –Day 1
- Veterinary Crush in Main Race and Veterinary Crush in 'Dip' Race scrubbed down with Hibitane.
- From 7:00 AM, 20 steers drafted off from the 63 steers for surgical implantation in the 20 ha paddock and walked quietly to the Main Yards
- 300 mm*300 mm area of the Right hand side para lumbar fossa clipped on each steer in the 'Dip' Race Veterinary Crush
- In the Veterinary Crush of the Main Race, Sirtrack temperature transmitter implanted surgically in each steer as per approved protocol
- Individual steers released into a wetted holding yard until 5 completed steers aggregate at which time the small group walked quietly to an 11 ha well pastured paddock adjacent to the cattle yards.
- If more steers are required for surgery on this day, an appropriate number of steers will be drafted off from the balance of steers awaiting for surgical implantation in the 20 ha paddock and walked quietly to the Main Yards. Clipping and surgery will be undertaken as above.
- Working cattle yards wetted and races/crushes hosed down (With a Gerni or Karcher if available) following completion of surgery

Wednesday October 10, 2007 – Surgical implantation of temperature transmitters –Day 2
- Veterinary Crush in Main Race and Veterinary Crush in 'Dip' Race scrubbed down with Hibitane.
- From 7:00 AM, 20 steers drafted off from the balance of steers awaiting surgical implantation in the 20 ha paddock and walked quietly to the Main Yards
- 300 mm*300 mm area of the Right hand side para lumbar fossa clipped on each steer in the 'Dip' Race Veterinary Crush
- In the Veterinary Crush of the Main Race, Sirtrack temperature transmitter implanted surgically in each steer as per approved protocol

- Individual steers released into a wetted holding yard until 5 completed steers aggregate at which time the small group walked quietly to an 11 ha well pastured paddock adjacent to the cattle yards.
- If more steers are required for surgery on this day, an appropriate number of steers will be drafted off from the balance of steers awaiting surgical implantation in the 20 ha paddock and walked quietly to the Main Yards. Clipping and surgery will be undertaken as above.
- Working cattle yards wetted and races/crushes hosed down (With a Gerni or Karcher if available) following completion of surgery
- The health and welfare of steers from Day 1 of surgery assessed by inspection

Thursday October 11, 2007 – If required to be conducted - surgical implantation of temperature transmitters –Day 3
- Veterinary Crush in Main Race and Veterinary Crush in 'Dip' Race scrubbed down with Hibitane.
- From 7:00 AM, any remaining steers awaiting surgical implantation in the 20 ha paddock walked quietly to the Main Yards
- 300 mm*300 mm area of the Right hand side para lumbar fossa clipped on each steer in the 'Dip' Race Veterinary Crush
- In the Veterinary Crush of the Main Race, Sirtrack temperature transmitter implanted surgically in each steer as per approved protocol
- Individual steers released into a wetted holding yard until 5 completed steers aggregate at which time the small group walked quietly to an 11 ha well pastured paddock adjacent to the cattle yards.
- The health and welfare of steers from Days 1 and 2 of surgery assessed by inspection

Monday October 15, 2007 – Veterinary inspection of 63 surgically implanted steers
- Working cattle yards wetted and races/crushes hosed down (With a Gerni or Karcher if available)
- 63 surgically implanted steers walked from the 11 ha paddock to the yards
- Each steer inspected in the Veterinary Crush of the Main Race by the Veterinary Surgeon
- After inspection, the 63 steers walked to a 20 ha paddock adjacent to the cattle yards

Thursday October 25, 2007 – Removal of sutures from 63 surgically implanted steers
- Working cattle yards wetted and races/crushes hosed down (With a Gerni or Karcher if available)
- 63 surgically implanted steers walked from the 11 ha paddock to the yards
- Each steer inspected and external sutures removed while in the Veterinary Crush of the Main Race.
- After suture removal, the 63 steers walked to a well pastured paddock

Monday November 5, 2007 - Procedures and measurements carried out on individual animals in the Main Race of the cattle yards
- Test weight of liveweight scales
- Veterinary inspection by Dr Rod Howard of all steers with surgically implanted Sirtrack Temperature Transmitters
- After 7:00 AM 63 surgically implanted steers walked to the Main Yards
- Steers remain off feed and water while in yards
- Measure non fasted liveweight as a check weight for impending induction
- Return steers their paddock.

Sunday November 11, 2007 (Day -1) – Allocate steers. Procedures and measurements carried out on individual animals in the Main Race of the cattle yards
- Test weight of liveweight scales
- At 7:00 AM all steers (as 2 separate mobs) walked to the Main Yards
- Steers remain off feed and water while in yards prior to measurements

- Estimate US Body Condition Score (USBCS) using method of Herd and Sprott (1996)
- Estimate visual hip height
- Measure non fasted liveweight
- Check operation of temperature transmitters in surgically implanted steers.
- Collect 2*10 ml vacutainers of venal blood via puncture of the coccygeal vein of the tail from each of the 63 surgically implanted steers.
- Hold steers in yards with access to water during allocation procedure
- Hold steers in yards overnight with access to hay and water.

Monday November 12, 2007 (Day 0) – Draft steers to treatment pens. Procedures and measurements carried out on individual animals in the Main Race of the cattle yards
- In Veterinary Crush in Main Race, apply colour code eartags based on treatments
- Draft steers into Replicate groups
- Draft steers into Treatment groups
- Walk steers to Treatment pens
- Feed steers in the PM within their Treatment Pens

Wednesday December 12, 2007 (Day 30) - Procedures and measurements carried out on individual animals in the Main Race of the cattle yards
- Test weight of liveweight scales

From 6:00 AM, walk steers from Treatment Pens in blocks of 4 individual pens in order of Pen 22 to 3.
- Steers remain off feed and water while in yards prior to measurements
- Estimate US Body Condition Score (USBCS) using method of Herd and Sprott (1996)
- Estimate visual hip height
- Measure non fasted liveweight
- Return steers in individual pen order of Pen 22 to 3.

Friday January 11, 2008 (Day 60) - Procedures and measurements carried out on individual animals in the Main Race of the cattle yards
- Test weight of liveweight scales
- From 6:00 AM, walk steers from Treatment Pens in blocks of 4 individual pens in order of Pen 22 to 3.
- Steers remain off feed and water while in yards prior to measurements
- Estimate US Body Condition Score (USBCS) using method of Herd and Sprott (1996)
- Estimate visual hip height
- Measure non fasted liveweight
- Return steers in individual pen order of Pen 22 to 3.

Sunday February 10, 2008 (Day 90) - Procedures and measurements carried out on individual animals in the Main Race of the cattle yards
- Test weight of liveweight scales
- From 6:00 AM, walk steers from Treatment Pens in blocks of 4 individual pens in order of Pen 22 to 3.
- Steers remain off feed and water while in yards prior to measurements
- Estimate US Body Condition Score (USBCS) using method of Herd and Sprott (1996)
- Estimate visual hip height
- Measure non fasted liveweight
- Return steers in individual pen order of Pen 22 to 3.

Saturday March 1, 2008 (Day 110) - Procedures and measurements carried out on individual animals in the Main Race of the cattle yards
- Test weight of liveweight scales
- From 6:00 AM, walk steers from Treatment Pens in blocks of 4 individual pens in order of Pen 22 to 3.
- Steers remain off feed and water while in yards prior to measurements
- Estimate US Body Condition Score (USBCS) using method of Herd and Sprott (1996)
- Estimate visual hip height
- Measure non fasted liveweight
- Collect 2*10 ml Vacutainers of venal blood via puncture of the coccygeal vein of the tail from each of the 63 surgically implanted steers.
- Return steers in individual pen order of Pen 22 to 3.

Tuesday March 11, 2008 (Exit) - Procedures and measurements carried out on individual animals in the Main Race of the cattle yards
- Test weight of liveweight scales
- From 6:00 AM, walk steers from Treatment Pens in blocks of 4 individual pens in order of Pen 7 to 22, Pen 3-6 and Pen 2.
- Steers remain off feed and water while in yards prior to measurements
- Estimate US Body Condition Score (USBCS) using method of Herd and Sprott (1996)
- Estimate visual hip height
- Measure non fasted liveweight
- Draft steers as per Drafting and Livestock Loading Appendix. To be completed by 9:30 AM.

Daily observations
- Animal health and welfare of the 63 steers surgically implanted with temperature transmitters during the post operative paddock phase
- Animal health and welfare of all steers assessed routinely from 7:00 AM while in the feedlot
- Animal behaviour observations including location/position of the animal in the pen and whether feeding, drinking or lying recorded during the 12:00 PM observations
- Panting score and respiration rate assessed at 7:00 AM, 12:30 PM and 5:30 PM
- Water temperature in each trough recorded at 12:00 PM

High heat load event observations, measurements and sampling – to be conducted by UQ, Gatton personnel on at least one by 4 day period per month, preferably to coincide with predicted stress events (projected on Accumulated Heat Load Units (AHLU's)). Observations will be made by Obsevers outside the pen every 2 hours bewteen 0600 and 1800 h during such events. The observations/measurements are:
- visual observations of animal behaviour – location in pen, whether standing, lying, eating, drinking
- panting scores - at least every 2 hours between 0600 and 1800 h
- remote measurement of body surface temperature by infra red device
- collection of venal blood samples by puncture of the jugular or coccygeal vein from heat stress affected steers and a random sample of unaffected steers from the population of steers with Sirtrack digital intraabdominal temperature transmitters. It is projected that a maximum of 30 such steers would be blood sampled on any one measurement occasion of 4 days. Blood collection will not be undertaken where cattle are showing signs of severe heat stress. The selection of steers with Sirtrack digital Intraabdominal temperature transmitters for this sampling would be based on their core body temperature profile at the time, panting score and surface body temperature.

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Appendix

Exit Day – Drafting of steers and loading of steers onto Livestock Transports

Drafting (during animal measurements process 6:00 AM to 9:30 AM):
- Betaine steers from Pens 7-10 processed and drafted into Transmitter Steers (12) to Water Yard A and Non Transmitters steers (20). The 20 Non Transmitter steers returned to Pen 7.
- Betaine steers from Pens 11-14 processed and drafted into Transmitter Steers (12) to Water Yard A and Non Transmitters steers (20). The 20 Non Transmitter steers returned to Pen 8.
- Betaine steers from Pens 15-18 processed and drafted into Transmitter Steers (12) to Water Yard B and Non Transmitters steers (20). The 20 Non Transmitter steers returned to Pen 9.
- Betaine steers from Pens 19-22 processed and drafted into Transmitter Steers (12) to Water Yard Band Non Transmitters steers (20). The 20 Non Transmitter steers returned to Pen 10.
- The 12 Spare Pen 2 steers processed as a group, held in a side yard and drafted into the following as the Glycerol Pen steers are processed:

2 Spares to Pen 3
3 Spares to Pen 4
2 Spares to Pen 5
3 Spares to Pen 6
2 Spares with Transmitters to the Transmitter Steer group.

- The Glycerol treatment steers from Pens 3-6 processed as discrete Pen Groups. The 3 transmitter steers from Pens 3-6 drafted as follows:

Pen 3 Transmitter steers drafted to Water Yard A
Pen 4 Transmitter steers drafted to Water Yard B
Pen 5 Transmitter steers drafted to Water Yard A
Pen 6 Transmitter steers drafted to Water Yard B
The 6 remaining Non Transmitter steers returned to their original pens

Loading onto Livestock Transports
From 10:00 AM

Truck 1 – After the Telonics Receiver and aerial installed, load the 62 steers with Temperature Transmitters as per the following:

Transmitter steers (62 steers)

Deck	Front compartment	Middle and rear compartments
Top At random – Glycerol Pens 3&5, Betaine Pens 7-14 & 1 Spare	n=11 (Random from Water Yard A)	n=20 (Random from Water Yard A)
Bottom At random – Glycerol Pens 4&6, Betaine Pens 15-22	n=11 (Random from Water Yard B)	n=20 (Random from Water Yard B)

& 1 Spare		

Truck 2 – Load livestock deck compartments as per the following:

Non transmitter steers (57 steers)

Deck	**Front compartment**	**Middle and rear compartments**
Top shaded	Pen 3 (n=6) Pen 2 (n=2) – random spares	Pens 7-10 (n=20) – random (From Pen 7 Holding)
Bottom unshaded	Pen 6 (n=6) Pen 2 (n=3) – random spares	Pens 11-14 (n=20) – random (From Pen 8 Holding)

Truck 3 - Load livestock deck compartments as per the following:

Non transmitter steers (57 steers)

Deck	**Front compartment**	**Middle and rear compartments**
Top unshaded	Pen 5 (n=6) Pen 2 (n=2) – random spares	Pens 19-22 (n=20) – random (From Pen 10 Holding)
Bottom shaded	Pen 4 (n=6) Pen 2 (n=3) – random spares	Pens 15-18 (n=20) – random (From Pen 10 Holding)

10.4 Appendix 4 Temperature transmitter surgical implantation procedure

Yard preparations prior to surgery
To minimise dust contamination, the accompanying cattle yards were wetted daily in the week prior to surgery. No animals were allowed to reside in the adjacent feedlot pens prior to surgery. The veterinary crush, cattle races, pound and forcing yard were hosed down prior to the commencement of surgery on each day and all cattle movements on each day within the cattle yards restricted to animals underging surgery. The veterinary crush was scrubbed down and disinfected with Cetridine prior to surgery on each day. There will no other cattle movements in the cattle yards during the days on which surgery will be conducted. Tarpaulins will be erected adjacent to the veterinary race/crush area to block the prevailing wind.

Temperature transmitter sterilization
Prior to sterilization of the temeprature transmitters (Sirtrack, New Zealand) (approximatly 75 mm × 25 mm × 20 mm) a loop of sutre lumen was attached to small hole located at the base of the transmitter. A stainless steel hook was inserted through the suture lumen of the transmitter, the transmitter turned on and suspended in 1 – 2 % glutaraldehyde in a sterile 1 L jar for 15 minutes. Five transmitters were placed in the sterile jar at the one time. After 15 minutes, the transmitter were lifted out of the glutaraldehyde solution and washed several times with 70% ethanol to remove all traces of the glutaraldehyde. The transmitter was then given a final wash with normal saline, the stainlesss steel hook was then removed and the transmitters placed in sterile capped specimen jars until used. The same process was repeated for each transmitter. The transmitters were handled by the stainless steel hooks with sterile gloved hands.

Surgical procedure
The sugery was carried out by Dr Rod Howard from Roma Veterinary Clinic and Ms Sonya Fardell a veterinary nurese from The University of Queensland.

The surgical procedure used was based on an originally approved procedure previously used by CSIRO Rockhampton and further modified following the completion of Study SA 2005/11/70. A detailed record of animal ID, time of entry into the crush, time surgery commences and finished, drugs used (name, amount, exp. date, batch number and withholding period) was recorded. Any additional comments such as "animal is agitated" were also recorded.

A 30 × 30 cm area on the right paralumbar fossa of each steer was clipped using a #10 clipper blade prior to surgery. This was done to minimise any contamination by hair in the veterinary crush being used for surgery. All surgical instruments/materials used were disposable, with individual disposable sterile packs of suture material and needles used for each steer. All disposable items including gloves were changed for every steer.

When a steer was put into the veterinary crush used for surgery, the clipped area of the right paralumbar fossa was washed using dilute povidine iodine. An inverted ‘L’ block was applied using Ilium Lignocaine 20 (Lignocaine 20mg/ml). The surgical area was prepared and sterilised using Iovone (7.5 mg/ml PVP) surgical scrub, then methylated spirits and cetridine surgical wash by the veterinary nurse. While this was happening Dr Howard scrubed up and re-gloved.

The surgery procedure was as follows: An 8 – 10 cm incision was made through the skin. A small incision was made through the subcutaneous tissue and external abdominal oblique. A blunt dissection was made through the internal abdominal oblique and transverse abdominus muscles to the level of the peritoneum. A pocket between the internal abdominal muscle layer and the

peritoeum was made by blunt dissection. The digital intraabdominal temperature transmitter (Sirtrack, New Zealand) (of approximate size 75 mm × 25 mm × 20 mm) was removed from its sterile container and inserted through the incision and located in this pocket i.e. external to the peritoneum i.e. between the peritoneum and the internal abdominal muscle layer. The muscular layers were closed using #2 chromic catgut (absorbable) in a simple continuous pattern. The skin incision in all steers was closed with # 3 supramid (non absorbable) using a ford-interlocking pattern. The surgical area was then given a final clean with dilute iodine and be sprayed with chloromide.

All steers were given a prophylactic injection of Engemycin 100 (oxytetracycline 100mg/ml) intramuscularly at 10ml/100kg. This was repeated after 3 – 5 days if required.

Following completion of a steer's surgery, such steers waited in yards with access to water until a small group aggregated then walked as a group to adjacent paddock/s. This was repeated throughout the day to avoid completed steers waiting for a long period in the yards.

Upon completion of surgery on each day, the completed steers were walked (100 m) to a 11 ha grassed paddock adjacent to the cattle yards where they remained for the 5 day period until initial post operative veterinary inspection.

Post operative care

The steers were inspected daily for assessment of their health and welfare. During the 2 - 3 day surgery period, all steers that have been implanted were inspected by Dr Howard. At 4 – 5 days post surgery all steers were walked from the 11 ha paddock to the main yards and each steer inspected by Dr Howard. The steers were returned to a 20 ha paddock adjacent to the yards. At 14 – 16 days post surgery, all steers were walked from the 20 ha paddock to the main yards and sutures removed. Following removal of sutures the steers were walked to another well pastured paddock approximately 40 m from the yards.

Post operative treatments were conducted under veterinary supervision.

10.5 Appendix 5 Supplement mineral composition and their expected analysis

Control/Betaine Treatment Rations

Expected Nutrient Analysis (DM %)

			Star	t – Int1	– Int2	- Fin
1	Dry Matter	%	83.087	81.650	82.218	83.743
2	Protein	%	14.339	13.856	13.543	13.229
161	Eq Prot N %	%	1.004	1.226	1.623	1.793
10	NEm	Mcal/kg	1.807	1.900	2.002	2.100
9	NEg	Mcal/kg	1.177	1.259	1.350	1.435
157	ME MJ/kg	-	11.612	12.085	12.593	13.089
12	NDF	%	25.821	23.640	20.885	18.775
13	eff-NDF	%	17.343	13.447	10.153	7.695
166	Roughage	%	29.291	19.617	15.863	13.150
14	TDN	%	77.621	81.012	84.181	87.112
16	Vit A	IU/kg	2201.284	2688.047	3559.306	3931.278
18	Vit E	IU/kg	6.854	6.721	8.900	9.830
19	Calcium	%	0.776	0.689	0.803	0.834
20	Phosphorus	%	0.397	0.422	0.419	0.418
34	Ca:P Ratio	-	1.956	1.632	1.915	1.995
21	Salt	%	0.112	0.137	0.181	0.200
22	Potassium	%	1.378	1.096	0.879	0.710
23	Sulfur	%	0.275	0.263	0.257	0.246
24	Magnesium	%	0.256	0.242	0.220	0.200
37	CA Balance	-	7.870	4.069	1.901	0.489
151	Monensin	g/ton	12.444	15.195	20.120	22.223
41	Fat	%	3.421	3.671	4.847	6.182

Glycerol Treatment Rations

Expected Nutrient Analysis (DM %)

			Glyc - Start	Glyc – Int1	Glyc – Int2	Glyc - Fin
1	Dry Matter	%	82.942	81.448	82.054	83.569
2	Protein	%	14.360	13.835	13.637	13.273
161	Eq Prot N %	%	1.006	1.230	1.627	1.798
10	NEm	Mcal/kg	1.815	1.903	2.010	2.114
9	NEg	Mcal/kg	1.188	1.267	1.361	1.452
157	ME MJ/kg	-	11.706	12.163	12.699	13.231
12	NDF	%	25.554	23.402	20.628	18.283
13	eff-NDF	%	17.440	13.690	10.385	7.726
166	Roughage	%	29.343	19.875	16.102	13.178
14	TDN	%	73.699	77.006	80.240	83.346
16	Vit A	IU/kg	2205.135	2694.708	3566.405	3939.467
18	Vit E	IU/kg	6.866	6.738	8.917	9.850
19	Calcium	%	0.748	0.665	0.775	0.805

20	Phosphorus	%	0.388	0.409	0.408	0.404
34	Ca:P Ratio	-	1.928	1.625	1.900	1.992
21	Salt	%	0.138	0.155	0.187	0.201
22	Potassium	%	1.382	1.114	0.884	0.710
23	Sulfur	%	0.313	0.292	0.266	0.246
24	Magnesium	%	0.253	0.241	0.221	0.200
37	CA Balance	-	8.066	4.956	4.059	3.096
151	Monensin	g/ton	12.465	15.233	20.160	22.269
41	Fat	%	3.384	3.622	4.804	6.192

Supplement Ingredient Detail

Expected Nutrient Analysis (DM %)

			Betaine Treat Feedlot Supp (White Pack)	Glycerol Treat Feedlot Supp (Black Pack)
1	Dry Matter	%	94.493	94.191
2	Protein	%	42.597	51.627
3	RUP %Prot.	-	4.452	10.297
4	RDP %Prot.	-	95.548	89.703
5	SOL %Prot.	-	89.726	76.421
6	NPN	%	35.303	35.441
161	Eq Prot N %	%	35.303	35.441
7	ME	Mcal/kg	0.991	1.261
8	NEl	Mcal/kg	0.801	0.919
9	NEg	Mcal/kg	0.396	0.474
10	NEm	Mcal/kg	0.634	0.752
157	ME MJ/kg	-	4.147	5.276
11	ADF	%	4.400	3.737
12	NDF	%	13.875	9.189
13	eff-NDF	%	0.277	0.565
14	TDN	%	70.885	73.870
16	Vit A	IU/kg	77423.258	77671.109
18	Vit E	IU/kg	193.589	194.208
19	Calcium	%	14.366	14.381
20	Phosphorus	%	0.404	0.384
21	Salt	%	3.942	2.782
22	Potassium	%	0.716	0.827
23	Sulfur	%	1.850	0.167
24	Magnesium	%	0.810	1.115
25	Zinc	ppm	618.672	614.933
26	Iron	ppm	1361.905	1367.924
27	Copper	ppm	139.924	143.487

10.6 Appendix 6 – Supplement manufacturing and sample protocols

1. **Title:** Supplement manufacturing and sampling protocol for Betaine and Rumensin 100 recovery assay.
2. **Objective:** The objective of this study is to determine product recovery concentration of Betaine and Monensin in Blue, Pink Striped, Yellow, Red, Green, White and Black Striped bag treatment/feedlot supplements manufactured by Janos Hoey premix manufacturing plant in Forbes, NSW, Australia. A Project representative presence is required for mixing and sampling of all treatment supplements to obtain samples for submission to laboratory for product recovery assay.
3. **Method of Mixing Supplements at Janos Hoey premix manufacturing plant:** Scales used for supplement ingredient addition are to have been certified within the past 12 months. The mixing procedure conforms to Janos Hoey ISO 9001-2000 quality control standards.

   3.1. A copy of the supplement's batch sheet formula will be maintained for records.

4. **Treatment Materials and Methods:**

   4.1. **Betaine Active Pack Formula:** The formula for the Betaine Active Packs is provided on attached sheet (Table 1).

   The Active Packs will consist of the following:
   - The Blue – Control/Spares Betaine Placebo Pack will consist of 0.0g/t DM of Betaine
   - The Pink Striped – Glycerol Treatment Betaine Placebo Pack will consist of 0.0g/t DM of Betaine
   - The Yellow - Betaine10g Pack will consist of 71.3g/t DM of Betaine
   - The Red - Betaine20g Pack will consist of 141.7g/t DM of Betaine
   - The Green - Betaine40g Pack will consist of 279.8g/t DM of Betaine

   4.2. **Betaine and Glycerol Treatment Feedlot Supplement Formula:** The treatment feedlot supplement formula is provided on attached sheet (Table 2).

   The treatment feedlot supplements will consist of the following:
   - The White – Control/Betaine Treatments Feedlot Supplement will consist of 438.3g/t DM of Monensin
   - The Black Striped- Glycerol Treatment Feedlot Supplement will consist of 437.7g/t DM of Monensin

   4.3. **Procedures for Betaine Active Pack and Treatment Feedlot Supplement Manufacturing:**

      4.3.1. **Cleaning and Inspection:** Prior to the scheduled time for starting the mixing and sampling protocol, mixer, floor and surrounding area is to be thoroughly clean. A Project representative and plant manufacturing staff will visually inspect the area prior to manufacturing commencement.

      4.3.2. **Order of Mixing Supplements:** The load manufacturing sequence is listed below.

         4.3.2.1. **Batch A:** 900 kg - 000 kg/t Betaine - Placebo = Blue Bag, 120 Bags in 7.5kg Packs, Control/Spares Betaine Placebo

         4.3.2.2. **Batch B:** 600kg – 000kg/t Betaine – Placebo = Pink Striped Bag, 120 Bags in 5kg Packs, Glycerol Treatment Betaine Placebo

         4.3.2.3. **Batch C:** 600 kg – 67.3 kg/t Betaine = Yellow Bag, 120 Bags in 5kg Packs,

Betaine 10 Treatment

4.3.2.4. **Batch D :** 600 kg – 134.7 kg/t Betaine = Red Bag, 120 Bags in 5kg Packs, Betaine 20 Treatment

4.3.2.5. **Batch E:** 600 kg – 270.0 kg/t Betaine = Green Bag, 120 Bags in 5kg Packs, Betaine 40 Treatment

4.3.2.6. Mixer and transfer equipment require complete flushing as described in Janos Hoey flushing procedures

4.3.2.7. **Batch F:** 9900 kg - 4.22 kg/t Rumensin = White Bag, 220, 45kg bags, Control/Betaine Treatment Feedlot Supplement

4.3.2.8. **Batch G:** 2520 kg - 4.22 kg/t Rumensin = Black Striped Bag, 56, 45kg bags, Glycerol Treatment Feedlot Supplement

4.3.3. **Supplement Ingredient Addition:** Ensure all raw materials are consistent in physical character. The vertical mixer is to be charged with 50 % of carrier weight. All pre-weighed actives are to be added to mixer. Remainder of carrier is added to mixer. Mixer blending commences and continues for 5 minutes. After 5 minutes pre-weighed vegetable oil is added. Mixer blending continues for an additional 15 minutes. A 25 kg quantity is removed and return to mixer as described in Janos Hoey method of mixing.

5. **Treatment Sample Collection:** Betaine Active Pack and Treatment Feedlot Supplement sample collection for product recovery test.

5.1. Five, 250 to 300 g samples of Active Pack and Treatment Feedlot Supplements are obtained from sequentially filled bags by the Project representative. Sample collections removed during the filling of Batch A bags (Blue bags), Batch B (Pink Striped bags), Batch C (Yellow bags), Batch D (Red Bags) and Batch E (Green bags) include sampling at # 1, # 25, # 50, # 75, # 100 and # 120. Sample collections removed during the filling of Batch E (White bags) include sampling at # 1, # 45, # 90, # 135, # 180 and # 220. Sample collections removed during the filling of Batch F (Black Striped bags) include sampling at # 1, # 10, # 20, # 30, # 40 and # 56. Samples will be taken using a hand scoop from material of designated bags. The targeted sample size is approximately 1.5 kg to perform betaine and monensin sodium assays. The 1.5 kg sample is thoroughly mixed in a clean container, and then divided into three (3) sub samples – one sub sample for betaine assay, one sub sample for monensin sodium assay and one sub sample retained as a triplicate (large Ziplock bag). There will be one clean mixing container required per supplement. Triplicate samples are maintained by Project Representative.

Sample will be placed into a sample bag (large zip locked bag) and labelled with:

5.1.1. Supplement Retention Sample:
E.g. Blue, Control/Spares Betaine Placebo

5.1.2. Manufacturing Date:

5.1.3. Batch:
E.g. Batch A

5.2. Samples will be double bagged and placed into a rigid container and managed by Project representative for submission for product analysis.

5.3. The process will be repeated for each supplement.

Bagging and Labelling of Supplements: Batch A is to be bagged in 7.5 kg **BLUE** coloured bags. **Batch B** is to be bagged in 5kg **PINK STRIPE** coloured bags. **Batch C** is to be bagged in 5 kg **YELLOW** coloured bags. **Batch D** is to be bagged in 5 kg **RED** coloured bags. **Batch E** is to be

bagged in 5 kg **GREEN** coloured bags. **Batch F** is to be bagged in 45kg **WHITE** coloured bags. **Batch G** is to be bagged in 45kg **BLACK STRIPE** coloured bags.

6. **Supplement Storage:** The Active Pack bags of **Blue, Pink Stripe, Yellow, Red and Green** are stacked on a pallet and plastic wrapped. The Feedlot Supplement bags of **White** and **Black Stripe** are stacked on separate pallets and plastic wrapped. All pallets stored at Janos Hoey in appropriate storage area. Pallets not to be stored directly on top of each other. Dispatch and transport of supplements is to be confirmed by DPI&F (Qld) Brigalow Research Station.
7. **Product Recovery Evaluation:**
   7.1. **Criteria for Product Recovery Validation:** The mean concentration for Betaine supplied by Betaine manufacturer. The mean concentration for Monensin sodium must be within the analysis sensitivity (85% to 115% for Monensin sodium).
8. **Results:** Mean concentration, percent of mean theoretical concentration for Betaine and Monensin sodium will be reported for each batch of supplement.

Table 1 Betaine Active Pack Formulation:

Ingredient Specifications (AS FED)				
Ingredient (kg/t)	**Betaine Placebo (Blue & Pink Stripe Pack)**	**Betaine 10g (Yellow Pack)**	**Betaine 20g (Red Pack)**	**Betaine 40g (Green Pack)**
Cereal carrier	1000.00	932.70	865.30	730.00
Betaine 96%	00.00	67.30	134.70	270.00
TOTAL	1000.00	1000.00	1000.00	1000.00

Table 2 Control/Betaine and Glycerol Treatment Feedlot Supplement Formulation:

Ingredient Specifications (AS FED)		
Ingredient (kg/t)	**Control/Betaine Treatment Supplement (White Pack)**	**Glycerol Treatment Supplement (Black Stripe Pack)**
Cereal carrier	420.89	222.93
Soybean meal	-	244.00
Limestone	362.20	360.00
Urea	86.70	120.00
Ammonium sulphate	68.90	-
Magnesium oxide	10.00	15.10
Salt	35.10	26.20
Potassium chloride	4.44	-
ENC Beef-B	7.55	7.55
Rumensin 100	4.22	4.22
TOTAL	1000.00	1000.00

10.7 Appendix 7 - Bunk management, mixing and feed out protocol

Feed intake management and bunk allocation

- The Modified 'Clean Bunk at Midday' feed intake management program (Lawrence 1998) is to be used for the project.
- Any mass of daily feed residue remaining in a treatment bunk is estimated at the time of bunk allocation and retained in the bunk if not spoilt. If spoilt, the residue is weighed and discarded. If there is any evidence of ration sorting in a feed bunk over a 2 day period, to assist intake management, that feed bunk would be residued and cleaned out. Whenever a pen bunk is residued a sample of the residue is retained for dry matter determination.

Feed Wagon Mixer Scale Check and Load Tolerance Procedure:

The tractor drawn wagon mixer will have the scales checked and will adhere to the loading tolerances according to the following criteria.

- Feed Wagon Mixer Scale Checking Procedure: The Wagon Mixer is to be tested prior to commencement and approximately half way through the feeding period using a load test weight. The Wagon Mixer scales will be recalibrated if a one percent deviation (10 kg. maximum) exists. The scale check information is recorded for the Wagon Mixer.
- Feed Wagon Mixer Load Tolerance: Batches of final feed ingredients are loaded using a $\pm$ 5 kg tolerance for ingredients loaded with a front-end loader and a $\pm$ 1 kg tolerance for hand added ingredients. Unless otherwise specified other tolerances exist within normal site procedures.

Treatment rations – The treatment rations and their colour code are:

- T1. Control – nil Betaine - No shade - Blue
- T2. Control - nil Betaine + shade - Blue
- T3. Betaine - 10 g/hd/d Betaine - No shade - Yellow
- T4. Betaine - 10 g/hd/d Betaine + shade - Yellow
- T5. Betaine – 20 g/hd/d Betaine - No shade - Red
- T6. Betaine - 20 g/hd/d + shade - Red
- T7. Betaine - 40 g/hd/d Betaine - No shade - Green
- T8. Betaine - 40 g/hd/d + shade - Green
- T9. Glycerol - No shade - Pink
- T10. Glycerol + shade - Pink

Feed and supplement addition to Mixer: The Knight horizontal Mixer will be loaded in the following order:

- Wheat
- Feedlot Supplement
- Mix for 2 minutes
- Molasses
- Oil

- Mix for 2 minutes
- Active Treatment Supplement or Glycerol
- Mix for 2 minutes
- Cotton seed
- Silage
- Straw
- Mix until average straw length is in the range of 50-80 mm.

Ration mixing and feeding out

- The colour coded Treatment rations are to be mixed and fed out daily from late AM (?) to late PM (?). The T1/T2 ration will be fed to the Spares. The Treatment rations will be mixed and fed out in the following order:

Activity	Time
Bunk Call	12:30 PM
Ration T1/T2 & Spares (Blue)	2:00 PM
Ration T3/T4 (Yellow)	2:45 PM
Ration T5/T6 (Red)	3:30 PM
Ration T7/T8 (Green)	4:15 PM
Wash-out	
Ration T9/T10 (Pink)	5:00 PM
Wash-out	Day end

- At the start of mixing each day, the Mixer is assumed to be clean. The Mixer is cleaned and flushed with water at the nominated Wash-out times to ensure all residual ration is removed. Any surplus ration (overrun) from any treatment mixing or at the end of the feed out is to be discarded.

Feed Mixer Flushing Procedure:

The Feed Mixer Wagon will be flushed between some treatment ration mixes according to the following criteria.

- A ‘wagon flush’ procedure using a water pressure cleaner will be used to clean out the Mixer between the Mixing/Feedout of the T7/T8 (Green) and T9/T10 (Pink) treatment rations and at the end of the day after the T9/T10 (Pink)treatment ration. Approximately 100-150 litres of water will be used for this flushing process. All rinsate will be emptied onto the ground. The purpose is to eliminate the risk for any cross contamination of test products and/or rations. The Mixer will be visually inspected to determine cleanliness prior to mixing the next ration.

Mixing and feeding out data collection: As per the Brigalow Research Station ‘Feed Ticket’ Form adapted for the Project.

Ration sample collection: As per the Project ‘Ration sampling collection, drying procedure and dry matter determination’ Protocol.

Contingent procedure if incorrect Treatment ration delivered to a Bunk during Feed Out:

If the incorrect ration is delivered to a feed bunk during the Project, record on Feed Ticket, in Trial Diary and advise T Grant, I Loxton, or R Lawrence. The procedure to be followed is:

- **If steers in pen have or have not consumed any incorrect ration –** Remove steers from Pen immediately, remove all ration from the feed bunk and dispose. Clean the feed bunk. Feed out the correct ration and return steers to the Pen.
- Wash out the Feed Mixer Wagon using the above procedure and feed out the correct ration or re-mix and feed out the correct ration if required.

Supplement Disposal: Any unused remaining Feedlot supplement will be available to Brigalow Research Station to utilise. Any unused Active Treatment Supplement or glycerol will be returned to the University of Queensland, Gatton.

Reference

Lawrence, R.J. (1998). A comparison of feedlot bunk management strategies and their influence on cattle performance and health. *Proc. Aust. Soc. Anim. Prod.* **22**: 177-180

10.8 Appendix 8 Sirtrack temperature transmitter offset data

Mean (October, 2007 and April, 2008) temperature transmitter offsets

Sirtrack Frequency (mHz)	Temperature offset (° C)
150.100	0.63
150.120	0.76
150.140	0.91
150.160	0.61
150.180	0.71
150.200	0.84
150.220	0.74
150.240	0.67
150.260	0.62
150.280	0.86
150.300	0.78
150.320	0.93
150.340	0.57
150.360	0.62
150.380	0.79
150.400	0.94
150.420	0.63
150.440	0.80
150.460	0.60
150.480	0.65
150.500	0.63
150.520	0.85
150.540	0.72
150.560	0.76
150.580	0.64
150.600	0.72
150.620	0.78
150.640	0.62
150.660	0.58
150.680	0.90
150.700	0.58
150.720	0.67
150.740	0.71

150.760	0.56
150.780	0.88
150.800	0.71
150.820	0.77
150.840	Not recovered
150.860	0.56
150.880	0.56
150.900	0.64
150.920	0.71
150.940	0.75
150.960	0.77
150.980	0.70
151.000	0.79
151.020	0.65
151.040	0.80
151.060	0.71
151.080	Failed
151.100	0.64
151.140	0.57
151.160	0.62
151.180	0.65
151.200	Failed
151.220	0.74
151.240	0.56
151.260	0.49
151.280	0.60
151.300	0.65
151.320	0.67
151.340	0.59
151.360	0.81

10.9 Appendix 9 MLA Betaine/Shade Project - Brigalow Research Station - Loading/transit/unloading phase diary

MLA Betaine/Shade project – Brigalow Research Station

Loading/transit/unloading phase diary

Tuesday 11/03/08

Brigalow Research Station – loading phase

Climatic conditions during loading were clear and mild with ambient temperatures ranging from 23.7°C at 9:00 AM to 26.4°C at 10:30 AM.

0915 – Started loading **TRUCK 1 - 960.JHB**
Loading from front of trailer and filled **A** through to **F** (Top deck first, bottom deck second)

Prime Mover →

A **PEN 7** **10**	**B** **PEN 7** **10**	**C** **PEN 3** **8**
D **PEN 8** **10**	**E** **PEN 8** **10**	**F** **PEN 6** **9**

0929 – Loading of 960.JHB completed

0935 – Started loading **TRUCK 2 - 133.HSA**
Loading from rear of trailer and filled **A** through to **F** (Top deck first, bottom deck second)

Prime Mover →

C **PEN 9** **10**	**B** **PEN 9** **10**	**A** **PEN 5** **8**
F **PEN 10** **10**	**E** **PEN 10** **10**	**D** **PEN 4** **9**

0946 – Loading of 133.HSA completed

1005 – Started loading **TRUCK 3 - 576.GKI**
Loading from front of trailer and filled **A** through to **F** (bottom deck first, top deck second)

Prime Mover →

D TRANSMITTER WATER YARD A 10	E TRANSMITTER WATER YARD A 10		F TRANSMITTER WATER YARD A 11
A TRANSMITTER WATER YARD B 10	B TRANSMITTER WATER YARD B 10		C TRANSMITTER WATER YARD B 11

1018 – Loading of 576.GKI completed

LOGGER POSITIONING (TRUCK 3 {576.GKI})

All Hobo loggers (T1-T8, excluding T6) were placed at or slightly above head height of the steers, in positions to remain untouched by the steers.
Loggers were installed from 09:00 onwards with all loggers in place by 10:00

Prime Mover →

Top Deck – Rear Comp. T7	Top Deck – Mid Comp.	T1	Top Deck – Front Comp. T5
Bottom Deck – Rear Comp.	T2 Bottom Deck – Mid Comp. T8	T3	Bottom Deck – Front Comp. T4

Telonics receiver and aerial installed in Truck 3 {576.GKI} for collection of body temperature data of Transmitter steers during transit to Oakey Abattoir.

1035 – Finished weighing trucks (full weight) at weighbridge
1048 – Depart BRS

Transit phase
Trucks travelled in order of loading. Truck 1 (960.HSA) able to travel faster on Hwy than other two trucks and arrived at stop points and destination well before other trucks.

1135 – **STOP 1**
Glenmoral-Roundstone Rd (Just before Leichhardt Hwy)
All decks checked, none down

NEXT SCHEDULED STOP - WANDOAN

1235 – Taroom, no stop

1321 – **STOP 2**
Wandoan
TRUCK 1 (960.JHB) – 1 lying down with plenty of space
TRUCK 2 (133.HSA) – All standing
TRUCK 3 (576.GKI) – 2 lying down (3826, 3918)
1331 – Pulling out

NEXT SCHEDULED STOP - CHINCHILLA

1420 – **STOP 3**
Unplanned stop at Miles. Communication from Truck 3 (960.GKI) that Telonics Receiver had stopped beeping. External power source removed, and re-started in Data acquisition mode.
TRUCK 1 (960.JHB) was already past Miles and did not stop
TRUCK 2 (133.HSA) – All standing
TRUCK 3 (576.GKI) – 3 lying down (3826, 3918, 3986)

1431 – Pulling out

NEXT SCHEDULED STOP – OAKEY

TRUCK 1 – **STOP 3**
Scheduled stop at Chinchilla. (Unsure of time), but was approximately 5-10 minutes.
3909 lying down

1518 – **STOP 4** (ONLY TRUCK 2 {133.HSA} and TRUCK 3 {576.GKI})
Unscheduled stop at Brigalow – Police pulled over to check licence and log-books.
TRUCK 1 (960.JHB) was already past Brigalow and did not stop
TRUCK 2 (133.HSA) – 1 lying down (3838)
TRUCK 3 (576.GKI) – 1 lying down (3885)

1531 – Pulling out

NEXT STOP – OAKEY
Total transit distance was 420 km.

Oakey Abattoir - Unloading phase
1650 – Pulled off Warrego Hwy
1700 – Arrived at the loading ramp – Oakey Abattoir.

(TRUCK 1 {960.JHB} had already arrived and unloaded cattle)
Unloading order?
TRUCK 2 (133.HSA) unloaded cattle
Unloading order?
TRUCK 3 (576.GKI) unloaded cattle. Hobo loggers and Telonics receiver removed from Truck 3.
Unloading order – Top deck followed by bottom deck

Climatic conditions at arrival were clear and mild (25°C).
The steers were unloaded into 2 Shaded Receival Pens (one pen for the 62 Transmitter steers and another pen for the 114 Non Transmitter steers). At approximately 6:00 PM the steers were moved from the Receival Pens to the covered Shed Lairage Pens (Pen No. 8D for the 62 Transmitter steers

and Pen No. 16 for the 114 Non Transmitter steers). All steers had access to water in both Receival pens and Lairage pens.

10.10 Appendix 10 MLA Betaine/Shade Project – Abattoir processing – Oakey Abattoir March 12-13, 2008

Wednesday March 12, 2008

Lairage pens:
Steers had spent overnight from 6:00 PM on Tuesday March 11 in Lairage Pen 8D (62 Transmitter steers) and Pen 16 (114 Non Transmitter steers). Lairage pens were under cover with steel yard panels and concrete floors. Steers washed around 9:00 AM and 10 Spare steers drafted from the 114 Non Transmitter steers. Steers had access to water in the Lairage pens.

Overnight climatic conditions were clear and cool with a minimum temperature of 15.6°C.

Body temperatures of the Transmitter steers were recorded using the Telonics Receiver and aerial mounted on the Yards Office Observation Platform during the lairage period from approximately 6:00 PM on Tuesday March 11 until approximately 9:00 AM on Wednesday March 12.

Slaughter floor:
The steers were slaughtered between 9:54 AM and 11:54 AM in the order – 62 Transmitter steers, 104 Non Transmitter Steers and 10 Spare steers. The slaughter order within those groups was random. The slaughter process was programmed to occur without any breaks other than the routine 5 minute personnel break between the Slaughter Floor Smoko and Lunch session.

Due to an auger breakdown on the plant, stunning ceased at 11:04 AM for 20 minutes and recommenced at 11:24 AM. During that time a lunchbreak was taken by Slaughter Floor personnel. The bodies on the slaughter floor were not run off during this 20 minute break with sequence number bodies' #40-139 remaining hanging on the chain, thus a significant delay in those bodies reaching the chillers. Bodies 1-39 entered the Chiller prior to the 11:04 AM shutdown. Bodies 140-166 were slaughtered after the 20 minute break. There was an additional unplanned chain stoppage of 12 minutes between 11:35 AM and 11:47 AM.

Electrical inputs on the Slaughter Floor were:

Bleeding table immobiliser – 0 to 300 V, frequency 100-2550 Hz and pulse width of 50-200 msec.

Immobiliser settings on Back Stiffener at Hide Puller – 180 V (AC) frequency 50 Hz.

Electrical stimulation to accelerate pH decline was not used.

All carcase sides were hung by the achilles tendon.

Personnel & tasks

Slaughter sequence- Robert Lawrence, IAP
Carcase side tag application to left hand carcase sides – Tim Grant, DPI&F
Collect muscle tissue & subcutaneous tissue samples from 62 Transmitter Steers – Brian Leury, Melbourne Uni and omental fat tissues – Fahri Fahri and Althula, Vic DPI and Kristy DiGiacomo, Melbourne Uni.

Record liver and lung lesions – Nick Kempe, Feedworks
Collect Sirtrack Transmitters from right hand carcase side of 62 Transmitter steers – John Gaughan and Stephen Bonner, UQ, Gatton.
Measure pH declines on left hand sides of bodies - Sequence no's 1-39 and 140-166 – Janine Lau and Robert Strachan, MLA (MSA).

Chillers:
The first carcase side first entered Chiller 13 at 10:45 AM, with Chiller 13 loaded prior to Chiller 14 being loaded. The relative carcase side position on the chiller rails are shown in the chiller plan of Figure 1.

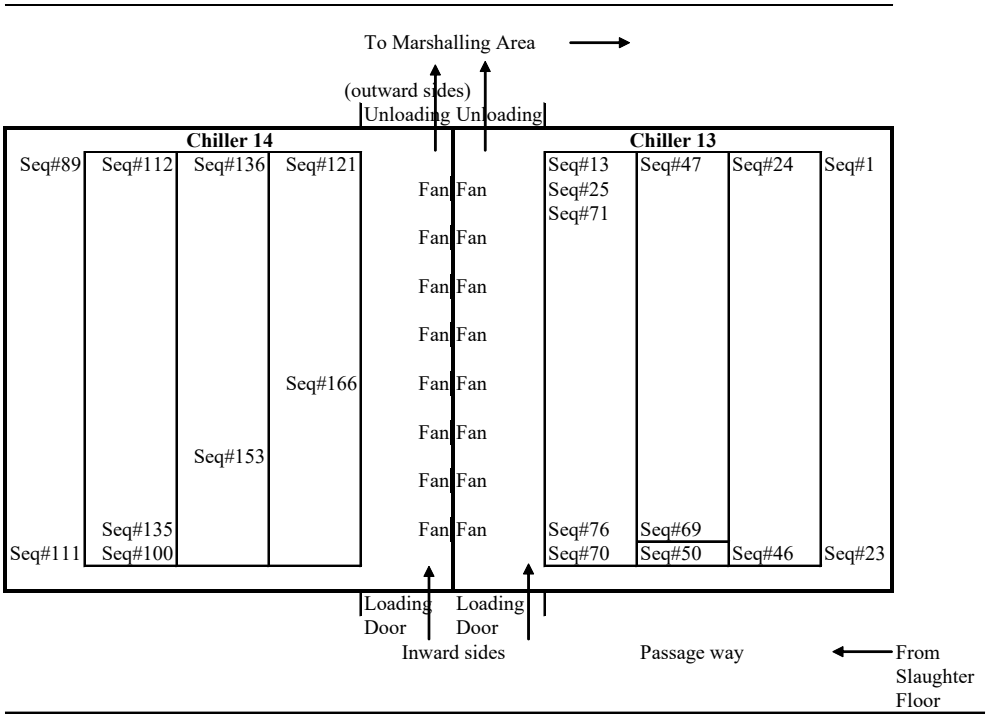

Figure 1. Plan of Chillers' 13 and 14 showing layout of rails and approximate positions of selected carcase sides (sequence numbers (Seq#n) on the rails.

Loading of Chillers' 13 and 14 was completed at 11:00 AM and 12:00 PM respectively and the chiller fans turned on at 12:00 PM and 1:00 PM respectively. The chiller fans were turned off around 3:00 AM on Thursday March 13. The duration of the chiller cooling cycle approximated 15 hours. The Chiller Temperature Management Program used is an approved modified program based on the 'Australian Standard for the Hygienic Production of Meat for Human Consumption (2nd Edition) which sets a target surface temperature of 7°C in 12 hours.

Thursday March 13, 2008

Chiller measurements:
The left hand carcase sides were marked at the 11/12th rib quartering point (2 rib hinds) from approximately 5:00 AM. Following marking the following measurements were taken between 5:30 AM and 9:30 AM:

Full MSA Grade – Left hand sides – 11/12th rib quartering point (2 rib hinds) – on 11th rib LD (i.e. cube roll). By Janine Lau and Robert Strachan, MLA (MSA)

LD Exudate/weep assessment – Left hand sides – 11/12th rib quartering point (2 rib hinds) – on 11th rib LD (i.e. cube roll). By Fahri Fahri and Althula, Vic DPI and Kristy DiGiacomo, Melbourne Uni.

LD HunterLab Miniscan Meat colour measurement – Left hand sides – 11/12th rib quartering point (2 rib hinds) – on 11th rib LD (i.e. cube roll). By Fahri Fahri and Althula, Vic DPI.

Striploin sample collection
Following the completion of all measurements in the Chiller, 200 mm long sections (approx. 1.75 kg) of the striploin from the quartering point were removed by an abattoir 'Slicer' from each left hand side. The striploin pieces were collected from the left hand carcase sides in Chiller 13 followed by the left hand carcase sides in Chiller 14, between 9:30 AM and 11:30 AM.

Each striploin piece (sample) was individually wrapped in plastic sheet and packed in cartons (10 samples/carton except for 1 carton of 6 samples) for a total of 17 cartons. The cartons of samples were weighed and strapped, then placed in the 'Plate Freezer' at 12:05 PM.

The cartons of samples were removed from the 'Plate Freezer' from 1:55 PM onwards on Friday March 14. The cartons were moved to a holding freezer until transport to Food Science Australia on Tuesday March 25. The cartons were transported from Oakey on March 25, 2008 in a refrigerated van (-10.0°C) leaving at 8:00 AM and arriving at Food Science Australia, Cannon Hill at 11:00 AM. The cartons were placed in frozen storage at arrival at Food Science Australia, Cannon Hill.

10.11 Appendix 11 Procedure for objective meat analysis

PROCEDURE FOR OBJECTIVE MEAT ANALYSIS

Receipt & Storage
The samples were delivered in a frozen state and placed in a -25°C freezer. A data logger was placed in a carton.

Thawing
48 hrs prior to the day of analysis, the samples were removed from the freezer. The samples were placed into plastic defrosting trays, ensuring that the samples were not touching and with the fat facing upwards. The trays were then placed into a 5°C chiller to allow the meat to thaw for 48 hours.

Preparation of longissimus dorsi (LD)
The muscles were removed from the 5°C chiller, placed into a 'Warwick' tray and stored in a 5°C chiller until selected for sample preparation.

A muscle was removed from the chiller, unwrapped, dried with paper towel and placed on the cutting board with the medial side (thick end or ‘backbone’ side) to the rear of the cutting board and with the fat upwards.

Fatty Acid analysis sample (for analysis by UQ)
A steak was sliced from the right-hand-side of the sample approximately 10-15mm thick through the subcutaneous fat and muscle. This was placed in a polyethylene plastic bag and labelled.

The fatty acid samples were then placed into in a large bag and vacuum packed and stored at -25°C until required for analysis.

Intramuscular fat analysis sample
A sample of LD (10mm thick) was trimmed of subcutaneous and intermuscular fat and taken to fat analysis (Thornton et al, Food Tech. Aust. 33, 468-473 (1981).

Sarcomere analysis sample
A 3-5mm thick sub sample was cut from the lateral side of the LD (thin end) of each sample.

Preparation of samples for objective measurements
The main sample was then trimmed to 250-260g ensuring that trimming was from the medial/back side and from the top or bottom of the sample. All samples were then placed on a marked tray in the 5°C chiller, covered and then left for 60 minutes for the samples to ‘bloom’ for colour measurements.

Objective Analyses

Meat Colour Measurement
After the samples had bloomed for 60 minutes, the tray was brought back into the laboratory. A colour measurement is taken on the bloomed surface using the Minolta Chroma Meter using light source C. Three measurements were taken and the average of the three readings recorded for L, a and b values.

pH readings
The pH meter was calibrated using BDH colour coded buffers pH 7.0 and pH 4.0. Four pH readings were then taken on each sample using a combination electrode (glass body with a spear tip). One temperature and 4 pH readings were taken for each sample.

Cooking and cook loss
The weighed samples were placed into plastic bags (255mm x 205mm x 70μm) labelled with treatment and identification code. The bags were folded, clipped into a holder, and placed into a preheated 70°C water bath for 60 minutes. The dimensions of the water bath were 90cm x 30cm x 32cm.

After cooking the samples were cooled in ice water for 30 minutes. The samples were then washed, dried, reweighed, placed and wrapped in a plastic bag and stored overnight in a 5°C chiller.

Preparation for Objective Assessment on Lloyd LRX testing machine
The samples were removed from the chiller. A sample is taken out of its bag and placed on a cutting board with the medial side to the left. A slice was taken from the lateral side to give a flat, vertical surface.

Warner-Bratzler Shear Test
See Perry *et al*. Aust. J Exp. Ag. 41, 953-957, (2001)

Compression Test (Texture Profile Analysis)
See Perry *et al*. Aust. J Exp. Ag. 41, 953-957, (2001)

FATS AND MOISTURES: SAMPLE PREPARATION BY DRYING
1. Homogenise sample of muscle (or fat) using Oskar blender
2. Weigh tins, ensuring each tin has two small round filter papers inside. Tabulate tin weights in correct column of data sheet. Tin weight plus papers will vary between 46 – 49g. Use two tins per sample unless triplicates are required.
3. Place approximately 15g of minced sample into each of the two tins and using the spoon, press sample evenly into a thin layer onto the filter papers. Ideally, weight of tin plus sample should be 63 - 65 grams
4. Replace the lid, and weigh tin + sample. Tabulate weights in correct column.
5. Remove lid, and place tins in drying oven. Place lids on side bench in order tins are placed in oven.
6. Set oven to 104°C for drying meat and fat, 65°C for drying fruit and vegetables.
7. Set timer for an 18 hour overnight drying cycle.
8. Next day, remove tins from oven and fit lids immediately. Do ten tins only at a time, and weigh again, tabulating results in the Dry Weight 1 column. Tins must be warm, not cold when weighing. Tins left too long will allow moisture from the air to be absorbed, giving inaccurate weights and results. Tins plus dried sample weight should be 50 – 53g approximately.

Calculating Chemical Lean Using Formulae
Four formulae for calculating chemical lean (CL) were supplied by Ian Eustace of Meat Industry Services. These formulae were taken from the Meat Technology Information Sheet “Microwave

Method for Chemical Lean Determination", published 1997 and reprinted November 2006 by Food Science Australia, AMPC and MLA.

Two of the formulae describe determination in beef, and one each for mutton and pork. Calculations of CL are only accurate for pure meat samples.

The beef CL calculation formulae are as follows:

For beef with an estimated CL of 80% or greater:

Chemical Lean (CL) % = (water x 1.21) + 5.44
For beef with an estimated CL of 79% or lower:

Chemical Lean (CL) % = (water x 1.35) – 3.2

Reference:
Perry, D., Shorthose, W. R., Ferguson, D, M. & Thompson, J. M. (2001). Methods used in the CRC Program for the determination of carcase yield and beef quality. *Australian Journal of Experimental Agriculture* **41**, 953-957.

10.12 Appendix 12 Procedure for assay of adipose tissue fatty acid profile

Adipose fat was used for analysis of fatty acid composition, derived from a sub-sample of *Longissimus dorsi* from which other meat quality parameters were assessed. A representative fat portion (approx. 20-30 mm) from the adipose fat depot was cut from the middle of the meat sub-sample, including all adipose fat external to the meat (within the 20-30 mm section). The cross-section of fat within the adipose tissue was thus represented within the fat portion for assay. The fat sample was thoroughly homogenised using a knife and spatula. The methanol-choloroform step for extraction of fatty acids from meat samples was not required within this analysis since the fat sample was obtained only from the adipose tissue. Trans-esterification of the fatty acids was conducted according to the following protocol:

(i) An internal standard solution was made by weighing out 100 mg of heptadecanoic acid (C17, margaric acid) into a 10mL volumetric flask, dissolved in AR iso propanol and diluted to volume.

(ii) Approximately 15-20 mg of fat sample was weighed into a clean 25 mL volumetric flask. A positive displacement pipette was used to add 100 µL of the internal standard solution.

(iii) Methanolic NaOH was added (0.5 mL of 0.5 M), the flask was flushed with N_2 and the stopper loosely fitted in the top.

(iv) The fat samples were saponified by placing on a steam bath at 95°C for 3-5 min only until all the sample was dissolved, avoiding taking the solvent to dryness.

(v) The samples were cooled and 2.5 mL of BF_3 – methanol solution (14%) added, the flask flushed with N_2 and the stopper reinserted loosely in the top. All flasks were placed in the water bath for 1 min with the stoppers inserted firmly.

(vi) The fatty acids were esterified by heating in the steam bath for 5 min, then cooled to room temperature.

(vii) 2.0 mL of heptane was added with a Gilson positive displacement pipette and mixed.

(viii) The saturated NaCl solution was added and the flasks agitated to mix the contents. Further solution was added to float the heptane up to the neck of the flask. Flasks were again stoppered and mixed.

(ix) Approximately 1.5 mL of the clear heptane solution was transferred into a 4 mL vial containing approx 100- 200 mg of anhydrous Na_2SO_4.after the phases had separated 1.0 mL was then transferred to a 2 mL Auto Sampler vial with a clean Pasteur pipette and the vial capped. Care was taken not to transfer particulates into the Auto Sampler vial.

Gas chromatography analysis of fatty acids
A Shimadzu gas chromatograph (Shimadzu Scientific Instruments, Rydalmere, New South Wales, Australia) was used with the following parameters:

- Column: J&W DB-Wax 30 m x 0.32 mm x 0.25 µm
- Injection temperature: 250°C
- Detector temperature: 285°C
- Column temperature: Start 100°C, 8°C/min to 250°C, hold 10 min, 5°C to 260°C hold 2 min
- Carrier gas Helium at 45 kPa linear velocity 20cm/s, pressure programmed for constant flow
- Sample injection 1 µL split ratio 15
- Auto Sampler set for 3 sample rinses followed after injection with 3 solvent rinses.

A standard curve was calculated using the peak areas obtained for the C17 internal standards between C17 concentration in µg/mL and peak area. Peak area was then converted into concentration calculated from the standard curve. Internal standard correction for loss in preparation was calculated using the ratio for peak area for C17 for each sample vs. the peak area for the C17 standard corresponding to 100 µL of standard. This ratio was used to correct each acid for loss in preparation and variation in injection volume.

10.13 Appendix 13 Heat stress management protocol

- Inspect cattle at 0600 h daily - if cattle are present with panting scores >1 a potential emergency situation may arise during the day.
- Management strategies (used exclusively or in conjunction with each other) that could be considered prior to a decision to withdraw imminently heat stressed cattle:

  - use overhead sprinklers to wet the cattle (NB Caution with sprinklers - wet muddy pens may exacerbate the problem - do not increase the moisture content of the pen surface of the pens especially if there is limited or no air movement and humidity in the pens is high). Cattle need to be wetted to the skin. Do not directly hose cattle in an attempt to wet them, as many cattle react adversely to being wet thus exacerbating their stressed state.

  - erect adequate temporary shade within the pen
- If cattle (particularly in un-shaded pens) become heat stressed during the day (up to 1900 h) (panting score 4; obviously distressed/agitated; deep shallow & laboured breathing, head down and drooling ceases) – open the pen gate allowing cattle to leave on their own accord (DO NOT FORCE THEM TO MOVE) to pens/yards that have shade However, any decision needs to be made in conjunction with weather conditions including the conditions that cattle have been exposed to over the last 24 hours. Cattle without respite over the previous 24 h are more susceptible to heat stress. If weather conditions are likely to worsen (e.g. HLI is going to increase in contrast to the previous day) then action may be required. However, if it is likely that conditions will abate within next couple of hours due to e.g. an increase in wind speed or a reduction in humidity then it may be prudent to take no immediate action in regards to moving or wetting cattle.
- A sudden drop in air movement with no associated abatement of hot conditions is a predisposing factor to heat stress. The critical factor in the management of heat stress affected animals is to do what is best for the animal, and act quickly.
- The animal is the best indicator of heat stress.
- Tim Grant will have the final decision on the implementation of strategies to manage heat stress after notification to and discussion with Ian Loxton/John Gaughan.
- Advise the relevant Veterinarian of the heat event/heat stressed cattle and document that the Veterinarian was advised.
- Any animal that was withdrawn from the project as a consequence of acute heat stress may be returned to its treatment pen following cessation of the heat load event or recovery by the animal from symptoms of acute heat stress.