POLKINGHORNE'S

MEAT & LIVESTOCK AUSTRALIA

final report

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Meat Standards Australia: Mixing and Stress Trial

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Abstract

These projects represented a major research effort to quantify the effect of stress imposed on animals through shipment by sea and through saleyard systems. Cattle from 4 Tasmanian and 4 King Island properties were processed through a Tasmanian abattoir with cuts collected for MSA consumer testing.

To create a range of stress, cattle were allocated to not mixed, mixed with the same sex or mixed sex groups from each property. Two forms of vessel and two saleyards were utilised with slaughter on arrival and after a 14 day rest period.

The direct delivery and non-mixed groups had substantially higher MSA compliance rates further reflected in moderately improved eating quality scores. Severe winter conditions during the second trial rest period emphasised that to be effective, recovery periods require active management with reference to prevailing conditions.

Several technologies with potential to measure individual animal stress were identified and utilised in the project including blood measures and non-invasive biometrics. Further work is required to bring these to an acceptable commercial level with each showing promise. Success would enable more accurate eating quality estimates and simplify MSA supply preconditions.

Three early career young scientists provided evaluation of blood (Kate Loudon from Murdoch University), infrared thermography (Holly Cuthbertson from Sydney University), and heart and respiration rate (Maria Jorquera-Chavez from Melbourne University) as major components of their PhD studies. The involvement of young researchers in a major industry research project is regarded as an ideal principal to provide valuable experience and encourage mentoring and engagement.

The outstanding assistance from the collaborating processor and all supply chain partners is acknowledged and greatly appreciated.

The project was conducted under approval R2839/16 of Murdoch University Animal Ethics Committee.

Executive summary

These projects represented a major research effort to quantify the effect of stress imposed on animals throughout two alternative supply chains. One supply chain included the transport of high quality slaughter cattle by sea and the other the movement of finished cattle through saleyard systems. Both were conducted in southern Australia with extensive cooperation and assistance from 8 beef properties located on King Island and in north western Tasmania. A major processing company provided outstanding assistance in recruiting suitable producers, managing all transport and providing on-site high quality resting paddocks through a difficult winter period.

There is strong industry demand to access MSA grading for all Australian cattle, documented by an MSA Taskforce objective to have all Australian cattle eligible for MSA grading by 2020. A further industry concern arose following the closure of the King Island abattoir requiring the sea transport of all slaughter cattle. The impact of voyage times and conditions on eating quality was not known and potentially could result in a large number of high quality cattle becoming ineligible for MSA grading. It became a high industry priority to establish whether a specific pathway was needed for shipping.

In response to these industry demands and considering the research background the MSA Pathways Committee proposed a trial that simultaneously attempted to answer questions about the relationships of stress and eating quality, and the potential measures of this, while also answering questions about shipping and saleyards pathways. It was agreed to design a highly robust trial to test various stressors and potential measures to identify and quantify animal stress pre-slaughter and the relationship, if any, between potential measures of stress and consumer rated eating quality.

The base research question was the impact of stress, created by transport, mixing and handling, on eating quality with related evaluation of potential objective measurement tools and management procedures to either reduce the impact or to recover prior to slaughter. To create a range of stress, cattle were allocated to not mixed, mixed with the same sex or mixed sex groups from each of 4 King Island properties and a further 4 in north western Tasmania. Cattle were sequentially allocated to treatment groups two or three weeks prior to loading with coloured eartags utilised to indicate treatment and trucking treatment. Crush score, flight speed, FLIR and retinal thermography was recorded for each animal to assess their capacity to predict stress. These measures were taken by MSA staff, young researchers and PhD candidates from Murdoch, Melbourne, Sydney Universities and the University of New England (UNE). The collaboration and involvement of young researchers in a major industry research project is regarded as an ideal principal to encourage mentoring and engagement.

On the exit day the mobs were penned into groups as indicated by eartag colour combinations and loaded to pre-allocated truck pens. Two forms of vessel were utilised from King Island, one a car carrier configuration in which two double deck trailers were loaded and a second where 4 double decks were unloaded to deck penning retaining the allocated groups.

For the Tasmanian saleyard phase the mixed treatment groups were trucked to two saleyards representing modern and traditional infrastructure with the never mixed groups trucked direct from farm to abattoir. Trucking was arranged to ensure a similar arrival time from all sources. At the

abattoir sub groups of each treatment were either programmed for kill or to a 14 day rest period in individual paddocks.

Thermographic images were taken of all cattle immediately pre-knocking and blood samples collected. Full pH and temperature declines were recorded in conjunction with MSA grading. Tenderloin, striploin, outside and oyster blade primal cuts were collected during boning and transferred to the University of New England for fabrication into MSA sensory samples. 7 and 21 day aged samples were prepared from all cuts and subsequently consumer tested.

The project succeeded in applying a varying degree of stress above pre-existing levels associated with cattle handling, loading, trucking and transit through a sea voyage or a saleyard environment. Extreme winter weather events added a further level during the second phase of the project. These events may in fact have limited the variation added by the project interventions.

The direct delivery and non-mixed groups had substantially higher MSA compliance rates further reflected in moderately improved eating quality scores. The rest period during the King Island phase was highly successful in dramatically increasing MSA compliance. In contrast the second phase rest period was ineffective and accompanied by severe winter conditions that impacted both the cattle held at the abattoir and those retained on farm although direct delivery of non-mixed groups again resulted in improved compliance.

This emphasised that to be effective, recovery periods require active management with reference to prevailing conditions.

The project also provoked detailed scientific and statistical consideration in regard to categorising individual animal rather than treatment group stress levels. While the treatments were designed, and were successful, in generating graduated additional stress challenges, the reaction of individual animals varied widely. Consequently response was not well categorised by comparing group means. No reliably effective means of categorising cattle of varying reaction was identified with options including blood metabolites, residual values from MSA model predictions, flight speed and behavioural measures and thermography all considered with none proving totally successful to this point.

Several technologies with potential to measure individual animal stress were utilised in the project including blood measures and non-invasive biometrics. This work was conducted within University PhD related programs and is, or will shortly be, reported in PhD thesis and through scientific publication. Kate Loudon, a PhD candidate at Murdoch University, was responsible for all blood analysis and has published her findings in two papers (Loudon et.al, 2019a, 2019b). Holly Cuthbertson from Sydney University conducted the infrared thermography (IRT) research component and has submitted four papers which are at the final review stage (Cutherbtson et.al, 2019a, 2019b, 2019c, 2019d). Maria Chavez, a PhD candidate at Melbourne University applied biometrics through video IRT presented a paper (Jorquera-Chavez et.al 2018) and poster at the Melbourne ICoMST meeting and has a further paper (Jorquera-Chavez et.al 2019) in advanced preparation.

While each technology showed promise and a general relationship to high pH and eating quality, none had the precision to provide an individual animal indicator suitable as an MSA grade input.

Further work is required to bring these to an acceptable commercial level. Success would enable more accurate eating quality estimates through including a stress indicator as a prediction model input. Identification of a reliable measure would also simplify MSA supply preconditions as direct assessment of the effect could replace mandated best practice requirements.

This would obviate the need to establish validated pathways that would render all cattle eligible for MSA grading. A substantial benefit to fixed pathways would be the capacity to factor in unique variables such as weather conditions or individual animal reactions. Hence there is strong interest in a single measure of stress that is practical, can be taken prior to slaughter and relate to any impact on eating quality as determined by consumer testing.

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1 Background

1.1 Project purpose

This project simultaneously attempted to answer questions relating to a presumed relationship between stress and eating quality and potential stress measures while also addressing industry demand for all cattle to be eligible for MSA grading whether processed through non-licensed saleyards, travelling long distances by road or transported by ship to slaughter.

This project aimed to generate sufficient data to validate various combinations of transport protocols and pathways that could underpin high quality beef. After extensive consultation, a robust trial design was agreed to test numerous stressors and potential measures to identify and quantify animal stress pre-slaughter and the relationship, if any, between potential measures and consumer rated eating quality. Experiments were approved and monitored by the animal ethics committee at Murdoch University under permit number R2839/16.

The base design employed variations of cattle mixing and transport to induce a range of stress impacts. Treatments comprised mixing of animals from different herds and sexes in addition to various transport treatments and pre-slaughter handling processes with further comparison of slaughter on arrival at the abattoir compared with a 2 week rest period prior to slaughter.

The design incorporated two phases to examine the effect of transport to abattoir via ship and transport to abattoir via saleyards. Phase 1 studied King Island cattle with treatments comprising mixing of animals from different herds plus sea transport to Tasmania followed by trucks to the abattoir and included comparison of a subset of cattle which were subject to a 2 week recovery period on pasture prior to slaughter. Phase 2 studied Tasmanian cattle with treatments comprised of mixing and saleyards induced stressors and comparison of a subset of cattle which were subject to a 2 week recovery period on pasture prior to slaughter.

The project evaluated potential stressors, remediation of stress, potential objective stress measures and their relationship to consumer tested eating quality. Measurements taken in the two phases were identical with the key difference being the transport pathway from farm to abattoir enabling data from both phases to be combined for statistical analysis and more rigorous consideration of the effect of different stressors, pathways to slaughter and potential measures of stress.

The project aimed to provide strong evidence based statistical input toward a review of MSA cattle supply criteria including sea transport, saleyard systems and standards regarding stock mixing and recovery pre-slaughter that could be successfully used by industry.

The project also provided a commercial platform for evaluation of emerging and existing technologies that might have potential to quantify animal stress related impact on eating quality. Success in this aspect had the potential to dramatically simplify MSA supply requirements while also improving eating quality prediction accuracy. A practical effective measure would offer potential to supplant existing delivery conditions while providing further input on handling effects on farm, during transit and at slaughter.

1.2 Project background

Consideration of stress and the desire to expand and quantify related eating quality effects is not new, having been considered an issue related to eating quality since the inception of MSA and prior. It has however been recognised as difficult due to the need for large cattle numbers, complex and diverse pathways and related cost to accurately confirm the multitude of interrelated potential factors involved. The 2011 background paper included in the appendix formed the basis for Pathways discussion of potential research approaches at that time. Subsequent MSA Pathways Committee meetings have further reviewed current knowledge, alternative research project designs and identified potential stress indicators or measures worthy of evaluation.

While ultimate pH has been used as a measure to reject carcases above 5.7 from MSA grading, studies by Warner et al. (2007) and Ferguson et al. (2008) reported that consumer assessed eating quality was detrimentally affected in some carcases where pH remained below 5.7.

Consequently eligibility conditions developed for MSA grading required cattle to meet a number of best practice related pre-conditions:

1. Cattle must be consigned by a licensed MSA producer and accompanied by MSA and NVD documentation.
2. Cattle to be consigned must not be mixed within 14 days of despatch from the property of origin including during transport and within lairage prior to slaughter.
3. Cattle that are sick, due to calve or in oestrus are excluded.
4. Slaughter must occur by the day after property despatch.
5. Saleyard cattle can be eligible but must be sold through an MSA licensed saleyard and meet conditions including no mixing and slaughter within 36 hours of property despatch. A 5 MQ4 point deduction is made to all model scores. Milk fed calves sold through a saleyard are not eligible.

These pre-conditions were established by the Pathways Committee to counter stress related conditions that were believed to be detrimental to eating quality but not able to be appraised during the grading process. It was recognised that excluded cattle could well include a proportion that exhibited acceptable eating quality but asserted that the variance and associated failure risk made a conservative risk based approach necessary to provide a consumer guarantee. Practical implementation by necessity required common treatment on a mob basis despite the likely variation in individual animal response.

While a MSA saleyard pathway was approved, it has not been widely adopted due to the demanding conditions imposed. A study by Polkinghorne et al. (2018) investigated long distance transport of 12, 24 and 36 hour duration in central western Queensland and concluded that transport time had little if any impact on ultimate eating quality but that there was a significant impact of handling on farm prior to and during loading. As a consequence MSA requirements were modified to allow trucking up to 36 hours and a farm to kill period of up to 48 hours. Many further combinations of pre-transport management, transport conditions including weather, intermediate rest and post transport management exist or are possible, however with each potentially requiring specific pathway criteria.

These conditions result in large numbers of cattle being ineligible for MSA grading. In the south, saleyard selling practices typically preclude MSA eligibility, while long distance transport and extended time from farm to slaughter exclude significant numbers in northern Australia. Further concerns relate to whether sea or rail transport differ in effect from road transport and the potential to remediate effects through rest and recovery post transport or mixing.

Another major background impetus to the current study has been industry demand, as reported in the Beef Language White Paper (2016) and through the MSA Beef Taskforce, for all cattle in Australia to have access to MSA grading. This includes cattle processed through saleyards in the south, cattle travelling long distances and times in the north and cattle being shipped. Following the closure of the King Island abattoir in 2012, there has been more specific concern about cattle that are shipped as opposed to trucked. These pathways represent significant numbers of cattle in Victoria, NSW and Tasmania that are currently ineligible for MSA grading and the associated potential higher returns to industry. In the north, long distances and combinations of road and rail plus resting and recovery practices remain largely untested.

The MSA Pathways Committee noted the need to investigate multiple areas including:

1. Stress detection either pre or during slaughter.
2. All possible measures that might be employed to measure degree of stress.
3. Quantification of the typical MQ4 effect of stress at various levels and inherent range.
4. Potential remediation/recovery Pathways.

The complexity of multiple pathways developed to address each combination with best management practices could be avoided if a single, individual animal measure of stress could be found. Warner et al. (2007) reported that despite Warner-Bratzler readings, pH decline rates and ultimate pH being similar for control and induced stress treatments (application of an electric prodder immediately prior to slaughter), consumer testing found a significant decrease in eating quality for the stressed treatment.

While multiple studies have been conducted relating blood metabolites to stress, none have produced practical, on-line commercial applications to quantify stress in relation to consumer assessed eating quality. Further, the industry ideal is to identify and rectify stress impacts prior to slaughter. Recent technological innovations in biometric measurement including FLIR and retinal scanning offer potential to assess animals prior to slaughter if stress or eating quality relationships can be established.

Success would be represented by developing the capability to replace mob based best practice requirements with direct individual animal measures that could be applied on a progressive scale, as currently done for *bos-indicus* content, and muscle specific basis within the MSA prediction model. Best practice management guidelines would subsequently become recommendations with grading representing the outcome.

In 2014, a project was developed by the MSA Pathways Committee to investigate and validate the effects of sea transport from King Island to Tasmania on animal stress including an evaluation of a post transport recovery period on pasture. The project proposed to also investigate packaging technology and saleyard impacts on eating quality and consumer sensory evaluation. Due to budget constraints it was not commissioned although the packaging aspect was addressed through an MDC project reported in P.PIP.0463.

The project design was reviewed within MSA 2015/16 research priorities and a final robust design agreed to assess multiple stressors, potential measures to identify and quantify individual animal stress pre-slaughter and the relationship, if any, to eating quality beyond current MSA grading inputs. This project, approved by the MSA Beef Taskforce, involved two phases, Phase 1 to evaluate shipping and mixing induced stress and the effect of a two week post transport rest period and Phase 2 in which the sea transport treatments were replaced by a comparison of mixing when processed through saleyards versus direct abattoir consignment. Evaluation of potential individual animal stress measures was overlaid on both phases through collaboration with Murdoch University (Blood evaluation), Sydney University (Infrared temperature FLIR measurement) and Melbourne University (Retinal scanning and biometry).

The project addresses questions relating to stress and eating quality relationships and potential individual animal measures while also developing data applicable to potential mob based shipping and saleyards pathways. It also complements investigations into low pasture magnesium and other pasture conditions being conducted on King Island cattle by Murdoch University.

1.3 Fundamental research questions addressed by the project

Does an ocean voyage from farm to slaughter require a specific MSA Pathway?

While MSA road transport time limits had been extended following research reported by Polkinghorne et al. (2018) there was MSA Pathways Committee and Industry concern as to whether road transport conditions related to ocean transport. The concern was upgraded when, as a result of the closure of the King Island abattoir, large numbers of high quality MSA eligible slaughter cattle required shipment by sea for slaughter in Tasmania.

Can a more industry acceptable MSA saleyard pathway be developed?

Industry demand for a less onerous MSA saleyard pathway remained strong with large numbers of young MSA eligible southern cattle not graded due to their marketing through saleyards. The existing pathway was regarded as too restrictive and further evidence regarding the impact of saleyard handling was required to inform potential change to MSA requirements.

Can stress be detected prior to or during slaughter?

From the MSA perspective the primary concern was that the consumer be protected through the identification of stress and grading or exclusion accordingly. However, from a processor and producer perspective, while protecting the consumer was critical, the detection of stress at slaughter / grading represented a preventable loss on a beast which became ineligible for MSA grading or was discounted. It would be of great value to producers and processors to have an objective measure of stress prior to slaughter so that identified stress could be prevented or remediated.

Considerable further value could be delivered if a similar measure could be utilised prior to slaughter enabling affected animals to be held and managed to remediate the problem thereby eliminating downgrades.

Can stress be remediated?

It was decided that the impact of a 14 day abattoir rest period on pasture should be tested within the project. If successful, this could provide a commercial option to render saleyard and other stressed or mixed cattle groups eligible for MSA grading.

What potential stressors should be considered?

It was decided that the following should be tested and anticipated that the combination would provide a range of stress response/s, ideal for evaluation of eating quality and objective measurement technologies.

- Mixing, of both same and mixed sex groups
- Transport methods
- The impact of pasture on farm prior to shipping and in post transport remediation

In light of technological development what measures should be tested?

Advances in technology had delivered new technology as well as new uses for existing technology that might be able to identify stress. Stress measures identified for evaluation were:

- Flight speed
- Crush score
- FLIR camera
- Retinal scanning as a means to measure heart rate, respiration rate and pupil dilation
- Blood parameters
- pH

Does any stress eating quality effect differ by muscle?

A hypothesis that “flight or fight” muscles may be more responsive to stress conditions than posture muscles, and further that post mortem ageing might be impacted was regarded as plausible. If stress related muscle differences were found this would impact MSA model adjustments, requiring individual muscle, and possibly muscle by ageing, adjustment rather than a “whole of animal” adjustment.

2 Project objectives

This project was developed through extensive consultation over a period of time and funded via two separate projects – L.EQT.1601 and L.EQT.1618. It is useful to note the agreement reached and recorded in MSA Pathways Committee minutes of 12th December 2015:

"It was agreed that the objective of MSA stress studies was to seek MQ4 effects related to stress and not explained by current MSA model inputs including pH. It was noted that pH was not well proven as an MQ4 indicator or predictor. At a broader level success in this objective was seen as facilitating MSA grading of all cattle due to removing the need for all existing MSA pre-slaughter Pathway requirements. (It was recognised that these would remain critical but the onus for best practice would rest with the supply chain). An agreed summary was "any at slaughter measures that can improve eating quality prediction beyond current model inputs".

The contract for L.EQT.1601 stated the following objectives:

In brief the trial design calls for two voyages on separate ships with a recovery component each two weeks apart. A total of 244 cattle are required for the split voyages with 61 steers sourced from each of two farms per voyage and 61 heifers from a further two farms per voyage. It is planned to record baseline stress measures and apply trial eartags two weeks or more prior to shipment. A number of cattle from each farm are to be shipped on the small boat to Stanley with the remaining per farm loaded on a common double deck crate and shipped on the large boat to Devonport. Both voyages are to be on the same dates within each replicate. Half of each small ship consignment and all from the large ship will be slaughtered on the day of arrival and the balance slaughtered 14 days later after rest on pasture.

By coordinating the kill of the first rested group and the arrival kill from the complementary Saleyard Trial potential kill day effects are reduced and the statistical design is much stronger. Further statistical strength would be gained if it were possible for one or more farms to supply for multiple voyages.

This current research will further investigate recent findings from a low pasture magnesium trial being conducted on King Island cattle. The previous trial found that the incidence of dark cutting was not associated with the grass tetany index however was associated with pasture magnesium levels and thus requires additional research.

At slaughter a range of potential stress measures will be observed or taken including collection of blood (a 10ml tube) plus temperature.

MSA graders will measure representative pH and temperature declines and collect MSA grading data pre-boning the day after slaughter. A Hunterlab and Nix device will be used to assess the potential for objective meat colour measurements. Selected cuts will be collected at boning with laminated ID tags placed on the cut prior to vacuum packing. The collected cuts will be shipped to Melbourne overnight for fabrication by MSA in other facilities. Both objective tests and consumer evaluation will be conducted on the collected cuts.

The contract for L.EQT.1618 stated the following objectives:

The current MSA Pathway for Saleyard cattle requires them to be processed at the abattoir within 36 hours of despatch from the property of origin including the sale process. This limits the number of cattle eligible for the MSA program. Two separate saleyards will be evaluated through this project with over 244 animals sensory tested.

A total of 44 picks is required to complete this evaluation. The creation of 44 picks involves a team required to pick and post. This process involves 36 samples per pick, broken down into plate and consumer allocations. 36 samples, when cooked, becomes 360 samples with each sample being tested by 10 consumers. At the completion on this project the consultant will have achieved 44 picks, which equals 2,640 consumers testing 1,584 individual samples.

3 Methodology – Phase 1 Collection

3.1 Trial Design – Phase 1

Phase 1 of the trial was designed to induce stress through mixing and transport, to measure the stress impact on eating quality as determined by consumers and to attempt to remediate the induced stress through rest with the ensuing eating quality also determined through consumer testing as per the Standard MSA protocols (Gee et al. 2005) and summarised by Anon (2008).

The design utilised 244 cattle from 4 replicate farms on King Island each supplying 61 cattle, with two farms supplying steers and 2 farms supplying heifers. Treatments were devised to create variable stress impact through mixing of cattle across farms and within and across sexes with control groups of steers and heifers that were never mixed. Table 1 details the allocation to treatments and vessels, with two double deck truck crates on vessel 1, and kill day in the final design.

Table 1. Number of head per farm and allocation by treatment and vessel related to kill day

TREATMENT MIX BY FARM, VESSEL and KILL DATE

	NEVER MIXED Steers		NEVER MIXED Heifers		MIXED STEERS		MIXED HEIFERS		MIXED SEX		
	Kill day 2	Kill day 15	Kill day 2	Kill day 15	Kill day 2	Kill day 15	Kill day 2	Kill day 15	Kill day 2	Kill day 15	TOTAL
VESSEL 1 - ORANGE TRUCK											
Farm 1	6				3				2		**11**
Farm 2	6				3				1		**10**
Farm 3			6				3		2		**11**
Farm 4			6				3		1		**10**
VESSEL 1 - GREEN TRUCK											
Farm 1	6				3				1		**10**
Farm 2	6				3				2		**11**
Farm 3			6				3		1		**10**
Farm 4			6				3		2		**11**
VESSEL 2											
Farm 1	8	8			8	8			4	4	**40**
Farm 2	8	8			8	8			4	4	**40**
Farm 3			8	8			8	8	4	4	**40**
Farm 4			8	8			8	8	4	4	**40**
TOTAL	**40**	**16**	**40**	**16**	**28**	**16**	**28**	**16**	**28**	**16**	**244**

Summary of allocation for one farm

	Never Mixed	Mixed Same Sex	Mixed Sex	Total
Vessel 1 - Orange truck	6	3	2	11
Vessel 1 - Green truck	6	3	1	10
Vessel 2	16	16	8	40
	28	22	11	**61**

Kill allocation

	Day 1	Day 2
Kill	**164**	**80**

The cattle were to be transported by truck from farm to the Grassy wharf on King Island and loaded onto 2 different ships. On arrival at Tasmania the cattle were to be transferred from ship to truck and driven to the abattoir. On arrival at the abattoir a sub set of each treatment group from each farm were designated to kills on the arrival day as per normal practice or after resting on good pasture for 2 weeks prior to slaughter.

Potential individual animal stress measures were prescribed at three time points with baseline measurements taken 2 weeks prior to transport, at the point of loading and the final measurements taken at the point of kill.

Specific measurements identified as potential measures of the relationship of stress and eating quality to be taken on farm during the initial handling three weeks prior to shipping were:

- Pasture and water measurements from the paddock being grazed.
- Weather station records from the Bureau of Meteorology.
- Continuous FLIR reading of cattle in the race leading to the scales and crush.
- A liveweight reading.
- A crush score allocated by observation of behaviour while held in the crush.

- Continuous FLIR reading of the eye area while restrained in the crush.
- Direct FLIR readings of the eye immediately after head restraint and again after eartagging and prior to release.
- A matching approximately 30 seconds of video retinal camera measurement on head restraint and after tagging
- Flight speed approximately 2 metres from crush exit

Cattle were to be sequentially allocated to treatment with surplus cattle drafted off during the initial handling. A combination of left and right eartag colours were to be utilised to visually indicate the allocated treatment and truck loading with the selected 61 head on each farm returned to pasture and managed as a single mob until transport. While the 61 head number was evaluated by power analysis to assess adequate sample size it was also influenced by the size of truck pens and the deck pen areas available on vessel 2. It was necessary to ensure that cattle of the weight selected were transported within appropriate welfare guidelines and to enable balanced numbers within treatments.

On the transport day the 61 selected animals were to be yarded and handled through the race and crush including head restraint without treatment. All live animal measurements, other than flight speed, were repeated. The cattle were drafted into allocated loading and treatment groups as indicated by eartag colour combinations.

Building on this foundation the specifics of the King Island scenario and commercial practice were taken into consideration, with transport components including the available trucks on King Island, the available ships and the trucks in Tasmania. The trial was initially designed to evaluate three ships, each of different configuration but was reduced to two due to the third vessel being removed from service. This required adjustment to the trial design to ensure statistically valid samples on each vessel and a penning design that would maintain required separate groupings as well as accounting for the effects of sea conditions across the vessel.

Vessel 1 was a moderate size “car ferry” operating a standard weekly overnight voyage from Grassy to Devonport with the capacity to directly load double deck cattle trailers in conjunction with other vehicles and general freight. The cattle trailers were loaded through the stern and remained below deck during the voyage. The trailers were spaced very close together within the hold and removed on arrival at Devonport where local prime movers were attached for the journey from port to abattoir. Consequently the cattle remained in the same trailer pen(s) from the farm until unloaded at the abattoir. Mixing treatments for cattle allocated to vessel 1 were to be achieved by sequential loading of two double decks at each of the four farms.

Vessel 2 sailed from Grassy to Stanley and had deck penning arrangements such that cattle were required to be loaded on a truck at the farm, driven to the wharf at Grassy, unloaded off the truck and moved into their new pens on the open deck in which they sailed to Stanley. On arrival in Stanley the research requirements dictated that they be moved off the deck and reloaded onto trucks for transfer to the abattoir retaining their original grouping. For vessel 2 four individual double deck crates and prime movers were specified to transport groups to the Grassy wharf with each truck designated to a single farm. Mixing treatments were to be applied by boxing designated truck pen groups from all 4 farms in the allocated shipboard pens.

The final design utilised two crates on vessel 1, a total of 84 head, with 160 head transported on vessel 2 in deck pens.

The experimental design required cattle from each farm, and each treatment group within farm, to be transported on both vessels within the same timeframe and for cattle to arrive at the abattoir within a common 2 hour time period. It was also stipulated that each treatment group be penned separately throughout the transport period and, to the extent possible, be distributed within the crate or shipboard pen in a controlled manner to balance potential position effects. This required a detailed loading instruction for each farm, for vessel 2 - pen allocation and for abattoir lairage - penning. Recording required during transport included time of loading and unloading at each point, weather and general sea condition observations. An observer travelled on vessel 2 to monitor animal behaviour during the voyage. Observers, including Dr Kate Loudon, a veterinarian from Murdoch University, were to inspect all groups during ship loading, on unloading from the vessels and on abattoir arrival.

The research design dictated that on arrival at the abattoir, cattle be unloaded in pen groups to maintain separation of treatment groups, and be subsequently allocated to lairage pens or holding paddocks in accordance with the trial design. All cattle from vessel 1 were allocated to slaughter on the day of arrival and maintained in their truck pen groups in lairage. Half of the vessel 2 cattle (80 head) were allocated to slaughter on the arrival day and half to a 2 week recovery treatment on pasture at the abattoir. The experimental design further specified a required kill order to ensure that treatments, source farms and vessels were distributed across the kill to minimise potential kill time effects.

Designated recording at the abattoir on each kill day included the following measures:

- Continuous FLIR recording from a camera mounted above the lead up race to the knocking box.
- A second continuous FLIR recoding from a camera mounted to the side of the lead up race.
- A third FLIR recording from a camera mounted directly above the knocking box.
- Three retinal scanning cameras mounted in the knocking box providing vision of both eyes from head restraint to knocking.
- Blood collection immediately post sticking.
- Eartag to carcase number correlation.
- Hourly pH and temperature readings from chiller entry for 5 hours to document pH and temperature declines in addition to ultimate pH.
- Full MSA grading data on the morning post slaughter.
- Muscle samples for glycogen assay.
- Pasture samples in the paddocks where rested cattle were to be held for two weeks.
- Weather records from abattoir arrival to final slaughter day.

An important trial design question was whether it could be assumed that an animal's response to stress is the same in all muscles or whether stress might display differently in muscles of different function such as posture muscles versus those used for flight or fight. To address this issue 5 muscles of divergent fibre type and function were designated for collection during boning:

- *M.psoas major*; Tenderloin TDR062, a posture muscle, unrelated to flight or movement.
- *M.longissimus dorsi lumborum*; Striploin STR045, believed to be principally a posture muscle but generally reactive to many treatments and MSA grade inputs.
- *M.biceps femoris*; Outside flat OUT005 and *M.semitenenosis*, Eeye round EYE075, both hind leg muscles and actively involved in flight.
- *M.infraspinatus;* Oyster blade OYS036, a shoulder muscle involved in locomotion and flight.

The trial design included provision of highly visual carcase identification tags and related coloured and uniquely numbered primal tags placed within the vacuum bags of each cut during collection.

Post collection, the primal cuts were cartoned and shipped using the standard abattoir refrigerated system which included initial refrigeration at the plant followed by loading the cartons in refrigerated containers for road transport to Burnie port, overnight sea freight to Melbourne and road delivery to a Melbourne refrigerated distribution facility managed by the abattoir.

The experimental protocol then required transport to the University of New England in Armidale for further fabrication into sensory and objective samples, ageing and allocation to consumer sensory sessions. To ensure prompt delivery and guard against loss of cuts, the trial design included use of a hired refrigerated transport vehicle for the transfer to Armidale.

Standard MSA protocols (Gee et al. 2005) were designated for preparation of consumer samples, ageing and subsequent consumer testing. To address potential stress interactions aging periods of 7 and 21 days were incorporated into the trial design.

3.2 Materials and Methods - Phase 1

3.2.1 Phase 1 - Scoping visit

A scoping visit was conducted by the Research Manager to better understand the typical commercial arrangements, and to discuss the proposed research design, associated requirements and to identify problems that would require design modification. The visit included meetings and discussion at the collaborating Tasmanian abattoir and with the abattoir representative and farm managers on King Island.

On site arrangements were managed by the abattoir, without whose cooperation the trial could not have been conducted. Abattoir management provided excellent background briefing on the supply system and local arrangements in relation to trucking and shipping practices. Initial discussion and onsite inspection at the abattoir confirmed practical aspects to manage the desired experimental protocols from arrival to cut despatch including management of cattle unloading, lairage penning, pasture available for multiple groups, kill planning, recording needs, boning, cut collection and transfer to Melbourne.

Abattoir management also recruited suitable farmer suppliers on King Island and agreed to manage trucking and ship coordination. Prior to visiting King Island, the local abattoir representative approached a number of suppliers and selected four who had appropriate cattle and suitable facilities for handling. Each farm was visited during the scoping visit and the design protocol discussed in detail. Farms were required to have sufficient numbers of single sex cattle such that they could supply 61 head of one sex on one day that would meet the abattoir specifications. Cattle yard facilities were also inspected at each property and management options discussed to facilitate the required drafting, holding and loading routines. It was required that the farms have reasonable standard yards with a scale and capacity to draft a minimum of 3 ways on the loading day, with additional draft capacity an advantage. A suitable loading ramp and all-weather access for single 40 foot trailer double deck trucks was required.

Review of the location of the four farms and unique layout of each set of yards informed the list of resources required for each farm visit taking into account factors such as:

- Cattle yards with no electricity available for the required equipment which necessitated the hiring of generators and required extension leads and connections.
- Potential locations and methods of securing the camera equipment to both wooden and steel yards in order to ensure the correct view and angle required to take the measurements of each beast as it went up the race, was held in the crush and as it exited from the crush.
- Photographs were taken for reference and a detailed list developed of required tools and supplies including G clamps, duct tape, wood screws, battery drills, extension leads, spare batteries, connections and general items. A minute level of detail was required to ensure all materials were available on the day with no window to source a replacement or back up materials due to a planned Sunday evening loading timetable and potential limited availability of required items on the Island at short notice.
- The yards' capacity to safely draft the cattle into the required lots to meet the penning design.
- Wet weather contingency options.
- Distance and truck travel times between farms and from farms to Grassy wharf.
- The number of trucks available and their typical multiple utilisation on a day that both vessels were to sail.

The interaction of trucking and ship loading was also discussed in detail and a deck plan of vessel 2 provided, together with an explanation of on board loading sequences. Detail of anticipated cattle liveweight in relation to truck and vessel 2 pen areas was critical in establishing suitable treatment numbers consistent with transport welfare guidelines and statistical requirements. A review of the available truck crate configuration and discussion with the truck drivers led to a revision of the draft loading plan to accommodate local practice of not transporting cattle on the crate ramp.

It was further established that there was no ability to draft cattle at the Grassy wharf or on vessel 2 requiring a detailed sequential loading plan and transport coordination across the four farms to enable the desired vessel penning plan to be achieved. It was agreed that sea conditions and swell could differentially affect cattle penned on the port or starboard sides or toward the stern or bow with the penning plan adjusted to as near as possible balance treatments across positions.

It was further ascertained that the two vessels normally loaded from the same wharf, with vessel two loading after vessel one was loaded and sailed, with a total loading window of two hours per vessel. This effectively dictated that cattle from the four farms be loaded simultaneously and transported to the wharf to ensure vessel loading in the available time. This necessitated having a research team on each of the four farms able to operate independently, with clear instructions for on farm teams and for truck drivers regarding the sequence of loading the trucks and the vessels. Detailed instruction sheets were prepared for each team, for the truck drivers and for the trucks transporting to the abattoir after docking in Devonport or Stanley in addition to designated lairage and holding paddock penning.

The ability to continuously identify the cattle and maintain their groupings was of paramount importance as was the ability to monitor the cattle at all stages of the handling and transport from the farm to the abattoir with a safe location for observers. Locally specific information gathered on the scoping visit influenced the final penning design and the tagging, drafting and loading for the trucks and the vessels to ensure the cattle were accurately identifiable at all times and moving through the trial in the required sequence and groupings.

A final outcome was confirmation of the number of people required to effectively manage each project segment, the coordination and local logistics for transport to King Island and from King Island to Tasmania to align with shipping timetables and associated freight arrangements for equipment, hiring of vehicles and accommodation arrangements. Lists were developed for all required equipment and consumables together with running sheets for each farm and standard sheets to record weights, scores and other data. Table 2 summarises the developed timeline for key activities.

Table 2. Phase 1 timeline - King Island stress and mixing trial

Timeline - KING ISLAND Stress & Mixing Trial		
DATE	**ACTION**	**ORGANISATION**
21-Apr	**Scoping trip** - King Island farms, trucking, vessels, abattoirs	PPL
27-Apr	King Island Trial - Travel to KI	PPL/MSA/Murd Uni/Syd Uni/Melb Uni
28-Apr	**Select & Tag cattle Farm 1 and 2**	
29-Apr	**Select & Tag cattle Farm 3 and 4**	
21-May	Final brief of participants on King Island	PPL
22-May	**Simultaneously Load cattle for trucking to Vessels**	PPL/MSA/Murd Uni/Syd Uni/Melb Uni
22-May	Cattle arrive at Abattoir from Statesman	PPL/MSA/Murdoch Uni
23-May	Cattle arrive at Abattoir from Mersey	PPL/MSA/Murdoch Uni
23-May	**King Island Trial - 1st Kill - Greenhams Abattoir**	PPL/MSA/Murd Uni/Syd Uni/Melb Uni
24-May	MSA Grading	PPL/MSA/Murdoch Uni
24-May	Boning/Cut Collection	PPL/MSA/Murdoch Uni
25-May	Ship to Greenhams Melb Depot	
26-May	Collect Depot Melb & Drive to Armidale	PPL
29-May	Cut up samples at UNE	PPL/MSA/UNE
30-May	Cut up samples at UNE	PPL/MSA/UNE
30-May	**Cut Up/Freeze down first (7 day aged) samples at UNE**	
5-Jun	Travel to Smithton - Cattle aready at Abattoir in holding paddocks	
6-Jun	**King Island Trial cattle - 2nd Kill - Greenhams Abattoir, Smithton**	PPL/MSA/Murd Uni/Syd Uni/Melb Uni
7-Jun	MSA Grading	PPL/MSA/Murdoch Uni
7-Jun	Boning/Cut Collection	PPL/MSA/Murdoch Uni
8-Jun	Ship to Greenhams Melb Depot	
9-Jun	Collect Depot Melb & Drive to Armidale	PPL
11-Jun	Cut up samples at UNE	PPL/MSA/UNE
12-Jun	Cut up samples at UNE	PPL/MSA/UNE
13-Jun	**Freeze down 7 day aged samples**	

3.2.2 Phase 1 - Research Team

A highlight of the project was the effective collaboration of a number of organisations and personnel within a complex and demanding research activity. The team assembled to deliver the field work was:

Rod Polkinghorne – Polkinghornes Pty Ltd – Research leader and manager

Judy Philpott – Polkinghornes Pty Ltd – Research logistics and coordination

Janine Lau – MSA Research & Development and Integrity manager – MSA coordination and project management (with further MSA grading staff involved on farm and in abattoir activity)

Jessira Perovic – MSA project administration and field work

Holly Cuthbertson – Sydney University PhD candidate – FLIR camera application

Kate Loudon – Murdoch University – Veterinarian – animal welfare, pasture and blood analysis.

Peter McGilchrist – Murdoch University – King Island field work and Kuchida (MIJ) camera

Maria Fernando Jorquera Chavez – Melbourne University PhD candidate – Retinal camera application

3.2.3 Phase 1 - Cattle selection, identification and baseline measurements

On the 28th and 29th April 2016 the project team travelled to King Island to select 61 cattle from each of the four farms in the trial, assign these cattle to treatments, identify them and gather the baseline measurements required before returning the selected 61 head to the paddock as a single mob.

Randomised treatment selection and identification

The source mobs were mustered by farm staff, rested for approximately one hour, then run through the handling yards at each property and sequentially drafted to a predetermined treatment allocation. To the degree possible the 61 head were selected to be uniform with cattle of divergent weight or type removed. Other than this the first 61 eligible cattle passing through the crush were selected.

Allocation of animals to treatment and vessel was determined prior to travelling to King Island with treatments sequentially allocated to crush entry order to ensure random distribution across the mob. Further pre-allocation to initial truck, vessel and kill date was made sequentially within the treatment allocations. A system of eartag colours was devised to facilitate on farm drafting pre-shipment.

The right ear tag colour corresponded to the ship – green and orange eartags for the two trucks, designated as “green and orange”, to be sequentially loaded from all farms with the crates then loaded on Vessel 1 and purple eartags for cattle to be loaded on the four trucks, one per farm, to be transferred to deck pens on Vessel 2. The left hand ear tag identified the treatment group and the farm. To generate sufficient unique colour combinations that would also be visible while safely drafting cattle some tags comprised a cattle tag of one colour with a different coloured sheep eartag punched into it so that there could be two colours in that ear. All eartags were numbered and assembled in pairs in tag order for each farm prior to travel to King Island. The relationship of eartag colour, treatment, truck from farm, vessel and kill designation is shown in Table 3.

Table 3. King Island tagging and allocation to truck, vessel and kill

Farm	Eartags	Treatment	Kill	No Head	Left Eartag	Vessel	Truck	Right Eartag
1	1 to 61	Never Mixed Steers	1	6	OLIVE & red	1	Orange	Orange
		Never Mixed Steers	1	6	OLIVE & red	1	Green	Green
		Never Mixed Steers	1	8	OLIVE & red	2		Purple
		Never Mixed Steers	2	8	RED	2		Purple
		Mixed Steers	1	3	Dark BLUE	1	Orange	Orange
		Mixed Steers	1	3	Dark BLUE	1	Green	Green
		Mixed Steers	1	8	Dark BLUE	2		Purple
		Mixed Steers	2	8	GREEN	2		Purple
		Mixed Sex	1	1	YELLOW	1	Orange	Orange
		Mixed Sex	1	2	YELLOW	1	Green	Green
		Mixed Sex	1	4	YELLOW	2		Purple
		Mixed Sex	2	4	ORANGE	2		Purple
				61				
2	62 to 122	Never Mixed Steers	1	6	OLIVE & blue	1	Orange	Orange
		Never Mixed Steers	1	6	OLIVE & blue	1	Green	Green
		Never Mixed Steers	1	8	OLIVE & blue	2		Purple
		Never Mixed Steers	2	8	OLIVE	2		Purple
		Mixed Steers	1	3	Dark BLUE	1	Orange	Orange
		Mixed Steers	1	3	Dark BLUE	1	Green	Green
		Mixed Steers	1	8	Dark BLUE	2		Purple
		Mixed Steers	2	8	GREEN	2		Purple
		Mixed Sex	1	2	YELLOW	1	Orange	Orange
		Mixed Sex	1	1	YELLOW	1	Green	Green
		Mixed Sex	1	4	YELLOW	2		Purple
		Mixed Sex	2	4	ORANGE	2		Purple
				61				
3	123 to 183	Never Mixed Heifers	1	6	WHITE & orange	1	Orange	Orange
		Never Mixed Heifers	1	6	WHITE & orange	1	Green	Green
		Never Mixed Heifers	1	8	WHITE & orange	2		Purple
		Never Mixed Heifers	2	8	LIGHT BLUE	2		Purple
		Mixed Heifers	1	3	PINK	1	Orange	Orange
		Mixed Heifers	1	3	PINK	1	Green	Green
		Mixed Heifers	1	8	PINK	2		Purple
		Mixed Heifers	2	8	WHITE	2		Purple
		Mixed Sex	1	1	YELLOW	1	Orange	Orange
		Mixed Sex	2	2	YELLOW	1	Green	Green
		Mixed Sex	1	4	YELLOW	2		Purple
		Mixed Sex	2	4	ORANGE	2		Purple
				61				
4	184 to 244	Never Mixed Heifers	1	6	WHITE & orange	1	Orange	Orange
		Never Mixed Heifers	1	6	WHITE & orange	1	Green	Green
		Never Mixed Heifers	1	8	WHITE & orange	2		Purple
		Never Mixed Heifers	2	8	LIGHT BLUE	2		Purple
		Mixed Heifers	1	3	PINK	1	Orange	Orange
		Mixed Heifers	1	3	PINK	1	Green	Green
		Mixed Heifers	1	8	PINK	2		Purple
		Mixed Heifers	2	8	WHITE	2		Purple
		Mixed Sex	1	2	YELLOW	1	Orange	Orange
		Mixed Sex	2	1	YELLOW	1	Green	Green
		Mixed Sex	1	4	YELLOW	2		Purple
		Mixed Sex	2	4	ORANGE	2		Purple
				61				

The detailed tagging sequence for each farm is shown in Table 4 with further explanation of the eartag coding below:

Table 4. King Island tagging detail by farm

FARM 1

Eartag No	Kill	Right Eartag	Vessel	Left Eartag: NEVER LARGE	Left Eartag: NEVER Small	Left Eartag: MIXED STEERS	Left Eartag: MIXED SEX
1	1	1	Vessel 2	1			
2	1	2	Vess 1 Or	2			
3	2	3	Vessel 2				3
4	1	4	Vess 1 Or			4	
5	2	5	Vessel 2			5	
6	2	6	Vessel 2	6			
7	1	7	Vess 1 Gr	7			
8	1	8	Vessel 2	8			
9	1	9	Vess 1 Gr				9
10	1	10	Vessel 2			10	
11	2	11	Vessel 2			11	
12	1	12	Vess 1 Or	12			
13	2	13	Vessel 2	13			
14	1	14	Vessel 2				14
15	1	15	Vess 1 Gr			15	
16	1	16	Vessel 2			16	
17	1	17	Vessel 2	17			
18	1	18	Vess 1 Gr	18			
19	2	19	Vessel 2	19			
20	2	20	Vessel 2				20
21	2	21	Vessel 2			21	
22	1	22	Vessel 2			22	
23	1	23	Vessel 2	23			
24	1	24	Vess 1 Or	24			
25	1	25	Vessel 2				25
26	1	26	Vess 1 Or			26	
27	2	27	Vessel 2			27	
28	2	28	Vessel 2	28			
29	1	29	Vess 1 Gr	29			
30	1	30	Vessel 2	30			
31	2	31	Vessel 2				31
32	1	32	Vessel 2			32	
33	2	33	Vessel 2			33	
34	1	34	Vess 1 Or	34			
35	2	35	Vessel 2	35			
36	1	36	Vess 1 Or				36
37	1	37	Vess 1 Gr			37	
38	1	38	Vessel 2			38	
39	1	39	Vess 1 Gr	39			
40	1	40	Vessel 2	40			
41	1	41	Vess 1 Or	41			
42	2	42	Vessel 2				42
43	2	43	Vessel 2			43	
44	1	44	Vessel 2			44	
45	2	45	Vessel 2	45			
46	1	46	Vess 1 Gr	46			
47	1	47	Vessel 2				47
48	1	48	Vess 1 Or			48	
49	2	49	Vessel 2			49	
50	1	50	Vessel 2	50			
51	1	51	Vess 1 Or	51			
52	2	52	Vessel 2	52			
53	1	53	Vess 1 Gr				53
54	1	54	Vessel 2			54	
55	2	55	Vessel 2			55	
56	1	56	Vessel 2	56			
57	1	57	Vess 1 Gr	57			
58	2	58	Vessel 2	58			
59	1	59	Vessel 2				59
60	1	60	Vess 1 Gr			60	
61	1	61	Vessel 2			61	
TOTAL		61		28		22	11

FARM 2

Eartag No	Kill	Right Eartag	Vessel	Left Eartag: NEVER LARGE	Left Eartag: NEVER Small	Left Eartag: MIXED STEERS	Left Eartag: MIXED SEX
62	2	62	Vessel 2	62			
63	1	63	Vess 1 Gr	63			
64	1	64	Vessel 2				64
65	1	65	Vess 1 Or			65	
66	1	66	Vessel 2			66	
67	1	67	Vessel 2	67			
68	1	68	Vess 1 Or	68			
69	2	69	Vessel 2	69			
70	1	70	Vess 1 Or				70
71	2	71	Vessel 2			71	
72	1	72	Vessel 2			72	
73	1	73	Vess 1 Gr	73			
74	1	74	Vessel 2	74			
75	2	75	Vessel 2				75
76	1	76	Vess 1 Gr			76	
77	2	77	Vessel 2			77	
78	2	78	Vessel 2	78			
79	1	79	Vess 1 Or	79			
80	1	80	Vessel 2	80			
81	1	81	Vessel 2				81
82	1	82	Vessel 2			82	
83	1	83	Vessel 2			83	
84	2	84	Vessel 2	84			
85	1	85	Vess 1 Gr	85			
86	2	86	Vessel 2				86
87	1	87	Vess 1 Or			87	
88	1	88	Vessel 2			88	
89	1	89	Vessel 2	89			
90	1	90	Vess 1 Or	90			
91	2	91	Vessel 2	91			
92	1	92	Vessel 2				92
93	2	93	Vessel 2			93	
94	1	94	Vessel 2			94	
95	1	95	Vess 1 Gr	95			
96	1	96	Vessel 2	96			
97	1	97	Vess 1 Gr				97
98	1	98	Vess 1 Gr			98	
99	2	99	Vessel 2			99	
100	1	100	Vess 1 Or	100			
101	2	101	Vessel 2	101			
102	1	102	Vess 1 Gr	102			
103	1	103	Vessel 2				103
104	1	104	Vessel 2			104	
105	2	105	Vessel 2			105	
106	1	106	Vessel 2	106			
107	1	107	Vess 1 Or	107			
108	2	108	Vessel 2				108
109	1	109	Vess 1 Or			109	
110	1	110	Vessel 2			110	
111	2	111	Vessel 2	111			
112	1	112	Vess 1 Gr	112			
113	1	113	Vessel 2	113			
114	1	114	Vess 1 Or				114
115	2	115	Vessel 2			115	
116	1	116	Vessel 2			116	
117	2	117	Vessel 2	117			
118	1	118	Vess 1 Or	118			
119	1	119	Vessel 2	119			
120	2	120	Vessel 2				120
121	1	121	Vess 1 Gr			121	
122	2	122	Vessel 2			122	
TOTAL		61		28		22	11

FARM 3							
				Left Eartag			
Eartag		Right		NEVER		MIXED	MIXED
No	Kill	Eartag	Vessel	LARGE	Small	HEIFERS	SEX
123	1	123	Vessel 2	123			
124	1	124	Vess 1 Or	124			
125	2	125	Vessel 2				125
126	1	126	Vess 1 Or			126	
127	2	127	Vessel 2			127	
128	2	128	Vessel 2	128			
129	1	129	Vess 1 Gr	129			
130	1	130	Vessel 2	130			
131	1	131	Vess 1 Gr				131
132	1	132	Vessel 2			132	
133	2	133	Vessel 2			133	
134	1	134	Vess 1 Or	134			
135	2	135	Vessel 2	135			
136	1	136	Vessel 2				136
137	1	137	Vess 1 Gr			137	
138	1	138	Vessel 2			138	
139	1	139	Vessel 2	139			
140	1	140	Vess 1 Gr	140			
141	2	141	Vessel 2	141			
142	2	142	Vessel 2				142
143	2	143	Vessel 2			143	
144	1	144	Vessel 2			144	
145	1	145	Vessel 2	145			
146	1	146	Vess 1 Or	146			
147	1	147	Vessel 2				147
148	1	148	Vess 1 Or			148	
149	2	149	Vessel 2			149	
150	2	150	Vessel 2	150			
151	1	151	Vess 1 Gr	151			
152	1	152	Vessel 2	152			
153	2	153	Vessel 2				153
154	1	154	Vessel 2			154	
155	2	155	Vessel 2			155	
156	1	156	Vess 1 Or	156			
157	2	157	Vessel 2	157			
158	1	158	Vess 1 Or				158
159	1	159	Vess 1 Gr			159	
160	1	160	Vessel 2			160	
161	1	161	Vess 1 Gr	161			
162	1	162	Vessel 2	162			
163	1	163	Vess 1 Or	163			
164	2	164	Vessel 2				164
165	2	165	Vessel 2			165	
166	1	166	Vessel 2			166	
167	2	167	Vessel 2	167			
168	1	168	Vess 1 Gr	168			
169	1	169	Vessel 2				169
170	1	170	Vess 1 Or			170	
171	2	171	Vessel 2			171	
172	1	172	Vessel 2	172			
173	1	173	Vess 1 Or	173			
174	2	174	Vessel 2	174			
175	1	175	Vess 1 Gr				175
176	1	176	Vessel 2			176	
177	2	177	Vessel 2			177	
178	1	178	Vessel 2	178			
179	1	179	Vess 1 Gr	179			
180	2	180	Vessel 2	180			
181	1	181	Vessel 2				181
182	1	182	Vess 1 Gr			182	
183	1	183	Vessel 2			183	
TOTAL		**61**		**28**		**22**	**11**

FARM 4							
				Left Eartag			
Eartag		Right		NEVER		MIXED	MIXED
No	Kill	Eartag	Vessel	LARGE	Small	HEIFERS	SEX
184	2	184	Vessel 2	184			
185	1	185	Vess 1 Gr	185			
186	1	186	Vessel 2				186
187	1	187	Vess 1 Or			187	
188	1	188	Vessel 2			188	
189	1	189	Vessel 2	189			
190	1	190	Vess 1 Or	190			
191	2	191	Vessel 2	191			
192	1	192	Vess 1 Or				192
193	2	193	Vessel 2			193	
194	1	194	Vessel 2			194	
195	1	195	Vess 1 Gr	195			
196	1	196	Vessel 2	196			
197	2	197	Vessel 2				197
198	1	198	Vess 1 Gr			198	
199	2	199	Vessel 2			199	
200	2	200	Vessel 2	200			
201	1	201	Vess 1 Or	201			
202	1	202	Vessel 2	202			
203	1	203	Vessel 2				203
204	1	204	Vessel 2			204	
205	2	205	Vessel 2			205	
206	2	206	Vessel 2	206			
207	1	207	Vess 1 Gr	207			
208	2	208	Vessel 2				208
209	1	209	Vess 1 Or			209	
210	1	210	Vessel 2			210	
211	1	211	Vessel 2	211			
212	1	212	Vess 1 Or	212			
213	2	213	Vessel 2	213			
214	1	214	Vessel 2				214
215	2	215	Vessel 2			215	
216	1	216	Vessel 2			216	
217	1	217	Vess 1 Gr	217			
218	1	218	Vessel 2	218			
219	1	219	Vess 1 Gr				219
220	1	220	Vess 1 Gr			220	
221	2	221	Vessel 2			221	
222	1	222	Vess 1 Or	222			
223	2	223	Vessel 2	223			
224	1	224	Vess 1 Gr	224			
225	1	225	Vessel 2				225
226	1	226	Vessel 2			226	
227	2	227	Vessel 2			227	
228	1	228	Vessel 2	228			
229	1	229	Vess 1 Or	229			
230	2	230	Vessel 2				230
231	1	231	Vess 1 Or			231	
232	1	232	Vessel 2			232	
233	2	233	Vessel 2	233			
234	1	234	Vess 1 Gr	234			
235	1	235	Vessel 2	235			
236	1	236	Vess 1 Or				236
237	2	237	Vessel 2			237	
238	1	238	Vessel 2			238	
239	2	239	Vessel 2	239			
240	1	240	Vess 1 Or	240			
241	1	241	Vessel 2	241			
242	2	242	Vessel 2				242
243	1	243	Vess 1 Gr			243	
244	2	244	Vessel 2			244	
TOTAL		**61**		**28**		**22**	**11**

Further explanation of the ear tagging follows:

Right Eartag Colour:

1	Purple indicated animal was allocated to **Vessel 2**. Right eartag colour and unique eartag number (same number as left) to identify each animal.
2	Orange indicated animal was allocated to **Vessel 1 on the Orange truck**. Right eartag colour and unique eartag number (same number as left) to identify each animal.
7	Green indicated animal was allocated to **Vessel 1 on the Green truck**. Right eartag colour and unique eartag number (same number as left) to identify each animal.

Left Eartag Colour:

- Column "NEVER" indicated group was not to be mixed with any other cattle from farm to kill. Left eartag colour and unique eartag number identified kill 1 or 2 with kill 1 cattle having a further small sheep tag with colour to signify farm.
- Column "MIX" indicated group was to be mixed with a group of the same sex from another farm during transport and kill. Left eartag colour and unique eartag number identified kill 1 or 2.
- Column "MXD SEX" indicated group was to be mixed with those from 3 other farms including both sexes during transport and kill. Left eartag colour and unique eartag number identified kill 1 or 2.

On farm implementation – Initial selection and base recording

The team comprised the abattoir King Island manager and representatives from Polkinghornes, MSA, Murdoch University, Melbourne University and Sydney University with cattle processed at Farms 3 and 2 on the 28th April, followed by Farms 4 and 1 on the 29th April.

The yards were set up with:

- An FLIR camera mounted above the race with mounting point and method adapted to suit each individual layout and provide a continuous clear view of cattle moving up the race.
- An FLIR camera mounted on a tripod just opposite and at right angles to the crush to enable a continuous picture to be taken of each animals head and eye while the head was held in the crush.
- Two flight speed sensors mounted at suitable positions after the crush in a position where they would not be damaged by the cattle. The position depended on the unique layout of each set of yards and was variously between 205 litre drums for protection, across a gateway, or between rails. The closest sensor to the crush was positioned more than 1 metre away to avoid breaking the beam during eartag application and retinal scanning. The distance between the two sensors ranged from 1,760 to 6,250 cm due individual yard layout considerations.
- Where necessary a portable generator was located at a distance from the yards to reduce noise and extension leads run as required.
- The property scales were checked for function with batteries attached where needed.

- A suitable area was located to lay out eartags, applicators and to record data.
- Access and position for the retinal camera operator was identified.

Each mob of cattle was mustered quietly to the yards by property personnel approximately an hour prior to handling. All cattle were moved through the race to the crush and any animal that did not meet the buyers specifications or that was not more or less uniform (ie; a Hereford from within a mob of Angus, or that was much heavier or lighter than the rest of the mob) was drafted off and removed from the trial group. On some properties a preliminary rough draft of surplus animals was conducted prior to the crush selection where initial numbers greatly exceeded requirements. The first 61 acceptable animals were selected, allocated to groups and tagged sequentially in the order of the pre-arranged tags.

FLIR thermal images were obtained of each animal moving through the race to the crush. Should the animal meet specifications, the animal's new eartag number was recorded. Should the animal be rejected then it was also noted within order to align FLIR output with animal ID (The FLIR cameras recorded heat gradient and consequently eartag numbers could not be distinguished in the thermal images).

Figure 1. Retinal camera (left) and FLIR image recording – Phase 2 yards showing the arrangement used in both Phase 1 and Phase 2

For each selected animal:

- Liveweight was recorded plus existing farm tags if applicable.
- A crush score was recorded on a 1 to 5 scale as proposed by Grandin (Grandin, 1993) with the scores assigned as:

  1 – calm, standing still, head mostly still or moving slowly;

  2 – slightly restless, looking around quicker, moving feet;

  3 – restless, moving back and forth in crush, shaking crush;

4 – nervous, continuous vigorous back and forth movement, snorting; and

5 – very nervous, continuous violent movements, attempting to jump from crush, snorting and vocalisation

- Retinal and FLIR thermographic readings were obtained for all selected cattle when caught in the crush head bail before touching eartags with time/image numbers recorded as appropriate to link animal ID to the image(s).
- Existing farm eartags were removed to avoid any confusion on the shipping day and details recorded.
- Each animal had two new eartags inserted in accordance with the treatment group as designated on worksheets and detailed in Tables 3 and 4.
- A second retinal and FLIR thermographic reading was recorded after the eartagging interaction.
- A continuous FLIR camera was utilised adjacent to the crush head bail to record temperature gradients while the head was restrained.
- Flight speed was recorded as the animal was released from the crush. Due to variation in distance between the sensors the time (seconds) between the sensors was adjusted for distance (cm) to determine the reported flight speed.
- The mob of 61 head was returned to pasture.

Figure 2. The arrangement used in both Phase 1 and Phase 2 for Retinal and FLIR readings taken in the crush

Figure 3. Tagged trial cattle returned to paddock

Running sheets and resources required for implementation may be viewed at Attachment C in the Appendix.

3.2.4 Phase 1 - Transport - King Island

The research design specified a two week rest period for all trial cattle after the visit for eartagging and baseline measurements. However, due to extremely rough weather the vessels did not sail as scheduled and the transport day was postponed to the 22nd May 2016, three weeks after the cattle selection visit.

Detailed recording, activity and instruction sheets were developed to control the on farm and wharf activity and provide detail of required actions and sequences for drafting, loading and transfer to vessel 2. Selected sheets were laminated to prevent damage with wet weather and included pictorial representations to clarify loading and penning requirements.

Four field teams were required to ensure cattle could be drafted and loaded from all 4 farms within the shipping timeframe. All personnel travelled to King Island together with required equipment and supplies the day prior to truck and vessel loading. Shipping and cattle truck bookings were made by the abattoir King Island representative who was a key resource in managing local arrangements and coordination. Each farm was visited to review the loading sequence and trial requirements the day prior to loading and a meeting was held with the transport operators to provide a briefing on trucking detail on farm and for unloading at the Grassy wharf. Two drivers who could not attend the meeting were visited later in the day and briefed.

Design of trucking and loading sequence

Six trucks were utilised on King Island with cattle loaded on three at each farm. The “Orange” and “Green” trucks were progressively loaded at all four properties and the two trailers then loaded in the hold of vessel 1. A further truck was fully loaded at each property with these four trucks unloaded and the cattle transferred to vessel 2 at the Grassy wharf.

The truck and vessel 2 loading sequences were detailed in laminated instruction sheets given to each farm team, with separate instructions also given to each truck driver and to those responsible for transfer from the four trucks to vessel 2. The order of loading for each farm was determined by truck arrival sequence and the penning design for each truck.

The “Orange” and “Green” truck designation aligned with the orange and green eartags in the right ear of the cattle groups assigned to those trucks. The Orange and the Green trucks loaded sequentially at all four farms in a predetermined order with cattle loaded on the truck in the sequence shown on laminated instruction sheets. At the Grassy wharf the trailers from the green and orange trucks were loaded in the hold of Vessel 1. They were subsequently unloaded and connected to other prime movers on arrival in Devonport, Tasmania, for transport to the abattoir.

Four trucks supplied Vessel 2 with each of these trucks collecting cattle with purple right eartags from one farm only. Vessel 2 required cattle to be loaded into pens on the deck and, as there were no drafting facilities at the wharf, cattle had to be loaded on the trucks in the reverse order of the ship penning. As there were four trucks supplying Vessel 2, each truck was scheduled to arrive and unload at the wharf in a specified sequence.

On farm component

As discussed, the loading schedule for the two vessels effectively required cattle from each farm to be loaded simultaneously, requiring a team of people at each of the 4 farms. The teams comprised representatives from Polkinghornes, MSA, Murdoch University, Melbourne University and Sydney University in addition to the King Island abattoir representative and farm personnel.

Individual worksheets and recording sheets for each farm team were prepared prior to travelling to King Island to muster, draft and load the cattle. Property personnel mustered the mob of 61 head to the yards which were prepared by the research teams with the cameras mounted as before. A continuous FLIR record was recorded of cattle moving through the race leading to the crush.

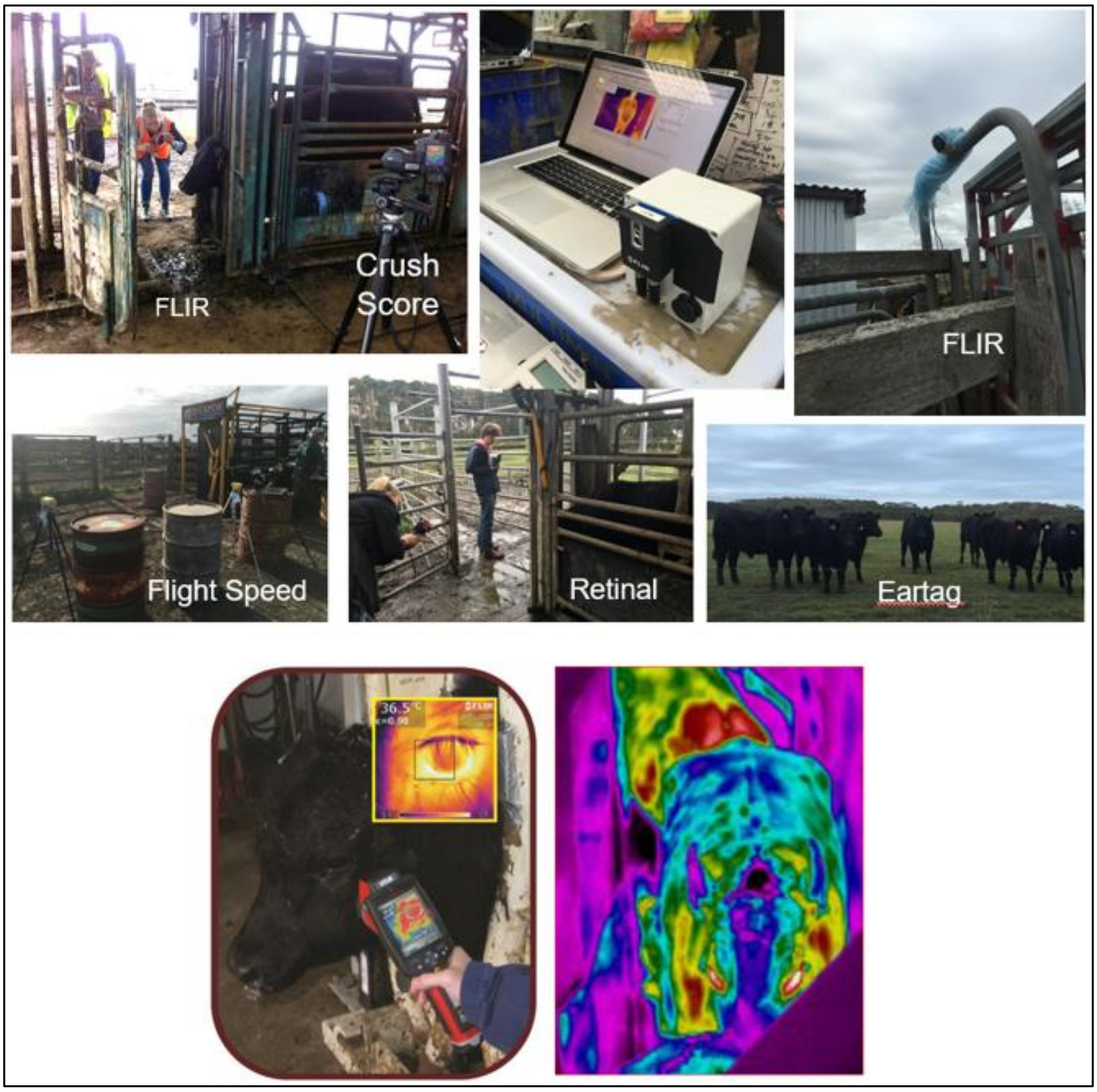


Figure 4. Pre-shipment recording

The recording sheets included an ordered check list of eartags for the specific farm to mark off and confirm all animals were present. Recording of eartags, weights and camera references were made on the sheet in order of animal presentation. Aside from convenience this was necessary to connect animal ID to the race mounted and crush head FLIR images. Eartags were checked with provision to replace any missing at this point. As there was no treatment applied in the crush only a single FLIR and retinal scan was required during head restraint and flight speed was not measured on this visit as there were not sufficient monitors to conduct this measurement at the 4 farms simultaneously.

The operational worksheet included a general check list to ensure the yards were operational for the required measurements and provided instruction for drafting on exit from the crush and subsequent further drafting, based on eartag colour combinations, required to achieve the designated truck loading sequence. The drafting instructions were specific to each property as the number of drafts available from the crush differed widely. Maximum use was made of all draft options at exit from the crush to minimise subsequent handling with pen from the crush, priority was given to “Never Mixed” groups. Further required sorting of the initial crush draft groups was conducted by property and research team personnel in the yards taking care to handle cattle as quietly as possible.

FARM 1 Worksheet for drafting and loading

Sunday May 22nd.

TEAM: Janine Lau, Maria Jorquera

Muster cattle to yards.
Set up camera on race.
Set up camera beside crush.
Check camera for retinal scan.
Check scale is functioning.
Weigh cattle and record weight with eartag in order weighed (needed for camera order).
Mark off eartag in Eartag Check column to ensure all are present.
Take retinal scan and record image No or time as required. (Note camera in heading)

ON EXIT FROM CRUSH:
DRAFT CATTLE INTO 3 GROUPS BASED ON RIGHT EARTAG COLOUR

GREEN EARTAG - right ear - 11 head to load on "Green" truck for Vessel 1
ORANGE EARTAG - right ear - 10 head to load on "Orange" truck for Vessel 1
PURPLE EARTAG - right ear - 40 head to load for Vessel 2

IN YARDS:
DRAFT GREEN RIGHT EARTAG STEERS TO 3 GROUPS BASED ON LEFT EARTAG COLOUR

GROUP 1 6 head with large OLIVE eartag and small RED sheep tag - left ear.
GROUP 2 3 head with large BLUE eartag - left ear.
GROUP 3 2 head with large YELLOW eartag - left ear.

DRAFT ORANGE RIGHT EARTAG STEERS TO 3 GROUPS BASED ON LEFT EARTAG COLOUR

GROUP 1 6 head with large OLIVE eartag and small RED sheep tag - left ear.
GROUP 2 3 head with large BLUE eartag - left ear.
GROUP 3 1 head with large YELLOW eartag - left ear.

DRAFT PURPLE RIGHT EARTAG STEERS TO 5 GROUPS BASED ON LEFT EARTAG COLOUR

GROUP 1. 4 head - large YELLOW tags - left ear.
GROUP 2. 16 head - 8 w large OLIVE & small RED sheep tag & 8 w large RED tags - left ear.
GROUP 3. 4 head - large ORANGE tags - left ear.
GROUP 4. 8 head - large GREEN tags - left ear.
GROUP 5. 8 head - large BLUE eartags - left ear.

Figure 5. Example of farm worksheet for drafting and loading

Truck loading sequences and specific truck pen allocation to drafted groups were communicated through printed and laminated control sheets provided to each farm and truck driver. As discussed previously the right eartag colour determined ship allocation and related truck. The left large eartag (and for never mixed groups additional small left eartag) colour designated group and related truck penning.

Research personnel were present in each farm team and during all handling treatments to record detail and oversee drafting and truck loading. Individual truck penning was strictly in accordance with the loading sheets provided to ensure treatment groups were allocated correctly to truck and ship penning. One steer exhibiting signs of severe metabolic stress during mustering and drafting at Farm 1 was not loaded on welfare grounds (Eartag 59 allocated to Mixed Sex treatment).

Figure 6 displays the "Green" truck instruction sheet with farm number substituted for property name. This sheet was provided to the truck driver and relates to the four progressive loading points.

KING ISLAND TRIAL - LOADING FOR VESSEL 1 - GREEN TRUCK

ALL cattle on this trailer will have a GREEN eartag in their RIGHT ear.

The truck will load at FOUR farms in this order - Farm 2 - Farm 3, - Farm 1 - Farm 4

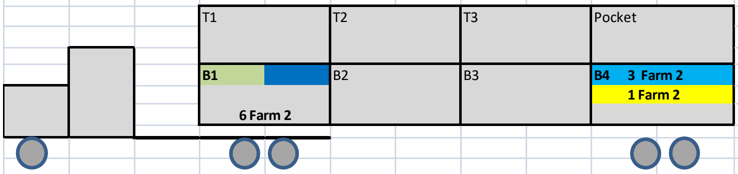
Tag colours below are for the LEFT ear

1. Truck to Farm 2

Load 6 steers (OLIVE with small BLUE sheeptag - left ear) to the front pen, bottom deck (B1)

**** If 6 steers are too tight - load 2 in the pocket

Load 4 steers (3 with BLUE eartag - left ear and 1 with YELLOW eartag - left ear) to an empty pen on the bottom deck



2. Truck to Farm 3

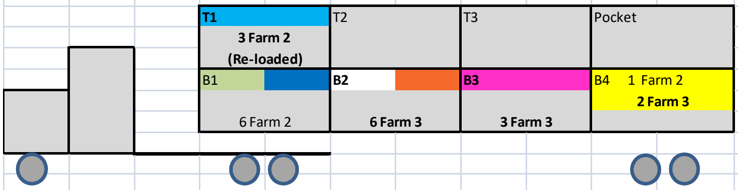
Unload 4 STEERS in bottom deck pen to the yards

Load 6 Farm 3 HEIFERS (WHITE eartag with small ORANGE sheep tag - left ear) to second pen on the bottom deck (B2)

Load 3 Farm 3 heifers (PINK eartag - left ear) to 3rd pen on the bottom deck (B3)

Lower the ramp and load 3 Farm 2 steers (BLUE - left ear) to the front pen on the top deck

Raise the ramp - load remaining Farm 2 steer (YELLOW tag - left ear) and 2 Farm 3 heifers (YELLOW tag - left ear) to the rear pen on the bottom deck (B4)



3. Truck to Farm 1

Unload 3 head (YELLOW eartags on left ear) from the rear pen on the bottom deck (B4)

Lower the ramp

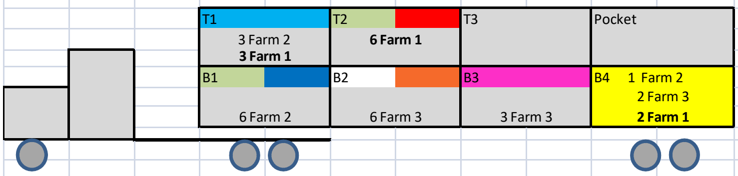
Box 3 Farm 1 steers (BLUE eartag - left ear) with the 3 Farm 2 steers (BLUE eartag- left ear) already in the front pen on the top deck

Load 6 Farm 1 steers (OLIVE with small RED sheep eartag - left ear) to the 2nd pen on the top deck (T2)

Raise the ramp

Box 2 Farm 1 steers (YELLOW tag - left ear) with 3 head already unloaded (YELLOW tag - left ear)

Load these 5 head to the rear pen on the bottom deck. (B4)



4. Truck to Farm 4

Unload 5 head (YELLOW tag in left ear) to the yards

Unload 3 Farm 3 Heifers (PINK eartag - left ear) from third pen on bottom deck to the yards and box with 3 Farm 4 heifers (PINK eartag - left ear)

Load 6 PINK eartag - left ear (3 unloaded Farm 3 heifers and 3 Farm 4 heifers) - all to be penned in 3rd pen on the bottom deck (B3)

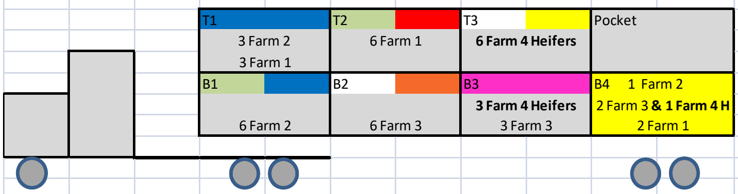
Lower the ramp

Load 6 Farm 4 heifers (WHITE eartag with small YELLOW sheeptag - left ear) to the 3rd pen on the top deck

Raise the ramp

Box 1 Farm 4 heifer (YELLOW eartag - left ear) with the 5 YELLOW eartags previously unloaded

Load 6 YELLOW eartags to the rear pen on the bottom deck (B4)



Drive to GRASSY for loading on Vessel 1

Figure 6. Loading instructions for the "GREEN" truck provided to farm teams and the truck driver

Instructions for the “Orange” truck were identical other than for the rear lower deck pen of 6 mixed sex cattle. Each property had either one or two animals in this pen on the “Green” truck and the reverse on the “Orange” truck for a total of 3 across the two trucks. Individual farm sheets were provided for each truck.

A similar loading instruction was provided for the four “PURPLE” trucks which each loaded at one farm only and proceeded to Grassy for cattle transfer to vessel 2. These sheets were property specific and supplied to the appropriate research team and truck driver. Figure 7 displays an example of truck loading for vessel 2 from one farm.

FIRST TRUCK FOR VESSEL 2

FARM 3

ALL cattle on this trailer (40) will have a PURPLE eartag in their RIGHT ear.
Tag colours below are for the LEFT ear

1. Draft PURPLE right eartags into 5 groups by LEFT EARTAG colour

GROUP 1.	4 head - large ORANGE tags - left ear.
GROUP 2.	16 head - 8 with large WHITE and small ORANGE sheep tag and 8 with LIGHT BLUE tags - left ear.
GROUP 3.	4 head - large YELLOW tags - left ear.
GROUP 4.	8 head - large WHITE tags - left ear.
GROUP 5.	8 head - large PINK tags - left ear.

2. Loading sequence

Load 4 ORANGE tags to front pen of bottom deck (GROUP 1) (B1).
Load any 6 of GROUP 2 (WHITE with small orange & LIGHT BLUE - left ear) to second pen on bottom deck (B2).
Load any 6 of GROUP 2 (WHITE with small orange & LIGHT BLUE - left ear) to third pen on bottom deck (B3).
Lower ramp
Load 4 YELLOW tags (GROUP 3) & any 2 of GROUP 4 (WHITE tags - left ear) to front pen of top deck (T1).
Load 6 WHITE tags - left ear (remainder of GROUP 4) to second pen of top deck (T2).
Load 2 PINK tags (GROUP 5) to rear of top deck (pocket).
Load 6 PINK tags (remainder of GROUP 5 to third pen of top deck (T3).
Raise ramp.
Load remaining 6 of GROUP 2 (WHITE and small orange or LIGHT BLUE - left ear) to rear pen of bottom deck (B4).

Drive to Grassy wharf.

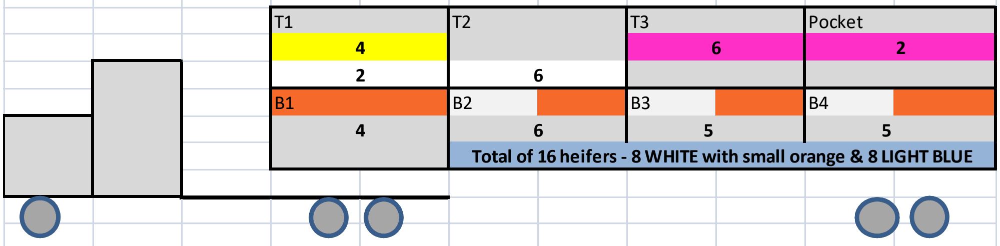


Figure 7. Truck loading instruction example for vessel 1 from property 3

3.2.5 Phase 1 - Transport - Shipping

On arrival at the King Island wharf the two trailers of cattle for Vessel 1 (Orange and Green right eartags) were loaded on board with other commercial cattle trailers. These were connected to new prime movers on arrival in Tasmania. As there was no ability for staff to access the hold during the voyage due to the close proximity of trailers and associated safety issues no observer travelled on Vessel 1.

The four trucks with cattle to be transferred to vessel 2 (Purple right ear tags) were unloaded in a pre-determined order to ensure a balance of deck position and treatment with the following final penning.

- Loaded 16 from Never Mixed pens to designated ship pen for each farm (Determined by left large and small eartag combinations) with kills 1 and 2 penned together to provide pens of 16.
- Loaded 8 Steers from Farm 1 and 8 from Farm 2 Mixed Steer groups for kill 1 (BLUE left eartags) and further 8 from each of farm 1 and 2 for kill 2 (GREEN left eartags). Penning indicated by left hand eartag colour.
- Loaded 8 Heifers from Farm 3 and 8 from Farm 4 Mixed Heifer groups for kill 1 (PINK left eartags) and further 8 from each of farm 3 and 4 for kill 2 (WHITE left eartags). Penning indicated by left hand eartag colour.
- Loaded two Mixed Sex pens with 4 head from each farm. Penning indicated by left hand eartag colour. Kill 1 YELLOW eartags and Kill 2 ORANGE eartags.

Figure 8 illustrates the deck pen configuration for vessel 2. Loading was conducted by connecting a portable ramp on the wharf between the truck and the central shipboard ramp which is rotated to connect to the wharf ramp. Figure 11 shows the equivalent arrangement during unloading at the abattoir.

The location of the shipboard ramp and pen relationship, size and construction had to be considered in allocating groups to specific pens of appropriate size and in endeavouring to balance treatments by port, starboard, stern and bow locations to minimise potential vessel position/ocean effects.

Figure 8. Pen configuration on Vessel 2

The final pen allocation for each cattle group is displayed in Figure 9. This pen design was then worked back to align the order of truck loading, unloading and movement of cattle to designated shipboard pens. Mixing treatments were applied by adding specified cattle from multiple farms to common pens with this visually indicated by left eartag colour and supported by documentation. To make up 16 head groups the first and second kill never mixed cattle groups were penned together for separation on abattoir arrival. All mixed groups totalled 16 within kill.

F	E	D	C	B	A	
			8 Farm 3 Heifers	8 Farm 1 Steers		
		16 Farm 2 Steers			16 Farm 3 Heifers	
			8 Farm 4 Heifers	8 Farm 2 Steers		
		8 Kill 1 - OLIVE & blue			8 Kill 1 WHITE & orange	
		8 Kill 2 - OLIVE	Kill 2 - WHITE	Kill 1 - DARK BLUE	8 Kill 2 - Light BLUE	M
G	**H**	**I**	**J**	**K**	**L**	
4 Farm 2 Steers	4 Farm 2 Steers		8 Farm 1 Steers	8 Farm 3 Heifers		
4 Farm 1 Steers	4 Farm 1 Steers	16 Farm 4 Heifers			16 Farm 1 Steers	
4 Farm 3 Heifers	4 Farm 3 Heifers		8 Farm 2 Steers	8 Farm 4 Heifers		
4 Farm 4 Heifers	4 Farm 4 Heifers	8 Kill 1 - WHITE & yellow			8 Kill 1 - OLIVE & red	
Kill 1 - YELLOW	Kill 2 - ORANGE	8 Kill 2 - PURPLE	Kill 2 - GREEN	Kill 1 - PINK	8 Kill 2 - RED	

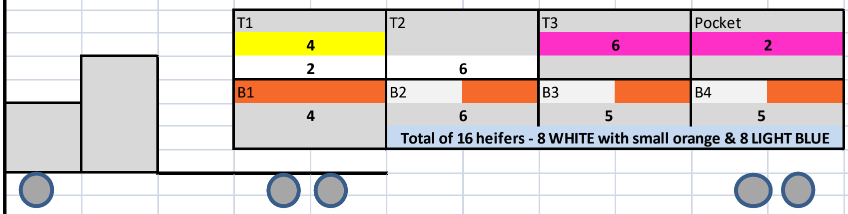
Figure 9. Vessel 2 penning design

Two pens in the stern (M) were excluded due to being too large for the trial group numbers and two port side pens near the bow as too small. To enable each truck to be fully unloaded without multiple movements to the ramp some pen groups were temporarily held in these pens (E and F) and then moved to their final pen after subsequent cattle from a later truck were positioned. The remaining pens, each suitable for 16 head, were allocated to achieve approximate balance across treatments. A loading sequence was then devised to achieve the desired penning within operational constraints related to loading sequence due to some pens being filled through other pens and others near the ramp needing to be filled last for OH&S considerations.

Further instruction sheets were developed and utilised to manage the unloading and shipboard penning as shown in Figure 10, below. After loading vessel 2 sailed for Stanley with an observer to monitor and record cattle and voyage detail.

VESSEL 2 LOADING SEQUENCE

FIRST TRUCK - FARM 3



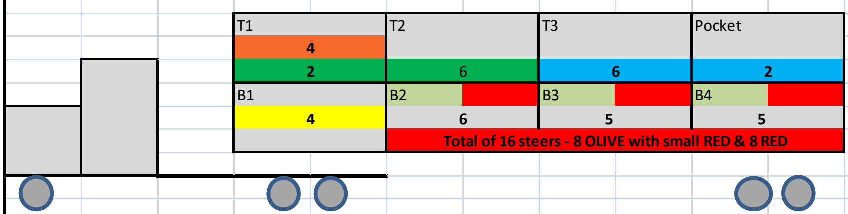
1. Unload rear 3 pens of bottom deck (16 head - 8 with large WHITE and small orange sheep tags and 8 with large LIGHT BLUE eatags - left ear) to **PEN A** on vessel
2. Unload front pen of bottom deck (4 head with large ORANGE eartags - left ear) to **PEN H** on vessel
3. Unload Top deck 3rd pen and pocket (8 head with large PINK eartags - left ear) to **PEN F** on vessel (temporary pen)
4. Unload top deck second pen (6 head with large WHITE eartags - left ear) to **PEN E** on vessel (temporary pen)
5. Unload top deck front pen - if possible separating 2 WHITE eartags - left ear from the 4 YELLOW eartags - left ear
6. WHITE and YELLOW left eartags to be drafted on truck or by holding on loading ramp to allow penning as required.
7. Add 2 WHITE large tags - left ear to previous 6 WHITE in **PEN E** (temporary pen).
8. Unload 4 YELLOW large tags - left ear to **PEN G** on vessel.

VESSEL PENNING AFTER FARM 3 TRUCK UNLOADING

F	E	D	C	B	A	M
8 Farm 3 Heifers	8 Farm 3 Heifers				16 Farm 3Heifers 8 Kill 1 WHITE & orange 8 Kill 2 - Light BLUE	

G	H	I	J	K	L	M
4 Farm 3 Heifers Kill 1 - YELLOW	4 Farm 3 Heifers Kill 2 - ORANGE					

SECOND TRUCK - FARM 1



1. Unload rear 3 pens of bottom deck (16 head - 8 with large OLIVE and small RED sheep tags and 8 with large RED eatags - left ear) to **PEN L** on vesse.l
2. Unload front pen of bottom deck (4 YELLOW large tags - left ear) to **PEN G** on vessel.
3. Unload Top deck 3rd pen and pocket (8 head with large BLUE eartags - left ear) to **PEN B** on vessel.

4. Move 8 heifers with PINK eartags - left ear from PEN F on vessel to PEN K.

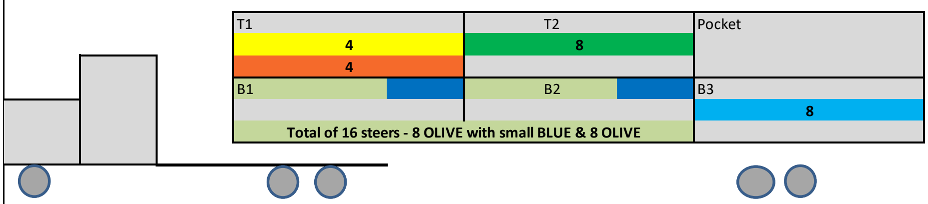
5. Unload top deck second pen (6 head with large GREEN eartags - left ear) to **PEN F** on vessel (temporary pen).
6. Unload top deck front pen - if possible separating 2 GREEN eartags - left ear from the 4 ORANGE eartags - left ear.
7. GREEN and ORANGE left eartags to be drafted on truck or by holding on loading ramp to allow penning as required.
8. Add 2 GREEN large tags - left ear to previous 6 GREEN in **PEN F** (temporary pen).
9. Unload 4 ORANGE large tags - left ear to **PEN H** on vessel.

VESSEL PENNING AFTER FARM 1 UNLOADING

F	E	D	C	B	A	M
8 Farm 1 Steers	8 Farm 3 Heifers			8 Farm 1 Steers Kill 1 - DARK BLUE	16 Farm 3 Heifers 8 Kill 1 WHITE & orange 8 Kill 2 - Light BLUE	

G	H	I	J	K	L	M
4 Farm 1 Steers 4 Farm 3 Heifers Kill 1 - YELLOW	4 Farm 1 Steers 4 Farm 3 Heifers Kill 2 - ORANGE			8 Farm 3 Heifers *(moved from F)* Kill 1 - PINK	16 Farm 1 Steers 8 Kill 1 - OLIVE & red 8 Kill 2 - RED	

THIRD TRUCK - FARM 2



1. Unload rear pen of bottom deck (B3) (8 head BLUE tag - left ear) and box with other 8 BLUE tag in **PEN B** on vessel.
2. Unload front and centre pens of bottom deck (B1 & B2) (8 large OLIVE with BLUE sheep tag & 8 large OLIVE tags) to **PEN D** on vessel.

3. Move 8 Farm 3 Heifers (large WHITE tag - left ear) in vessel PEN E to vessel PEN C.

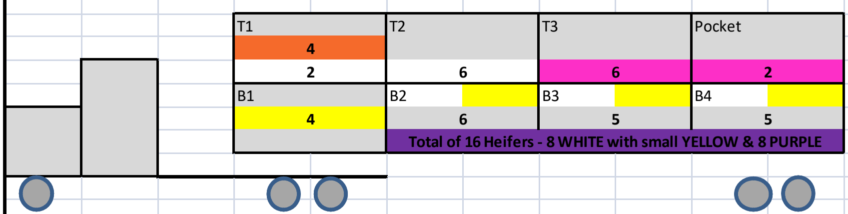
4. Unload top deck centre pen (T2) (8 GREEN tags - left ear) to **PEN E** on vessel (temporary pen).
5. *If possible sort off 4 large ORANGE tag - left ear in top deck front pen (T1) or hold as needed on ramp.*
6. *YELLOW and ORANGE left eartags in top deck front pen (T1) to be drafted on truck or by holding on loading ramp to allow penning as required.*
7. Unload 4 large ORANGE tag - left ear to **PEN H** on vessel.
8. Unload 4 large YELLOW tags - left ear to **PEN G** on vessel.

VESSEL PENNING AFTER FARM 1 STEERS

F	E	D	C	B	A	
8 Steers farm 1	8 Steers Farm 2	16 Steers Farm 2 8 Kill 1 - OLIVE & blue 8 Kill 2 - OLIVE	8 Heifers Farm 3 (moved from E)	8 Steers Farm 1 8 Steers Farm 2 Kill 1 - DARK BLUE	16 Heifers Farm 3 8 Kill 1 WHITE & orange 8 Kill 2 - Light BLUE	M

G	H	I	J	K	L
4 Steers Farm 2 4 Steers farm 1 4 Heifers Farm 3 Kill 1 - YELLOW	4 Steers Farm 2 4 Steers Farm 1 4 Heifers Farm 3 Kill 2 - ORANGE		Kill 2 - GREEN	8 Heifers Farm 3 Kill 1 - PINK	16 Steers Farm 1 8 Kill 1 - OLIVE & red 8 Kill 2 - RED

FOURTH TRUCK - FARM 4



1. Unload rear 3 pens (B2, B3 & B4) of bottom deck (8 large WHITE with YELLOW sheep tag & 8 PURPLE tag) to **PEN I** on vessel.
2. Unload front pen bottom deck (B1) (4 large YELLOW tags - left ear) to **PEN G** on vessel.
3. Unload top deck rear pen and pocket (8 large PINK tags - left ear) to **PEN K** on vessel.
4. Unload top deck second pen (6 large WHITE tags - left ear) to **PEN C** on vessel.
5. Unload top deck front pen - if possible separating 2 WHITE eartags - left ear from the 4 ORANGE eartags - left ear
6. WHITE and YELLOW left eartags to be drafted on truck or by holding on loading ramp to allow penning as required.
7. Unload 2 WHITE eartags and box with other WHITE in **PEN C** on vessel.
8. Unload 4 Orange eartags and box with other ORANGE in **PEN H** on vessel.

9. Move GREEN tags from vessel PEN F and PEN E to vessel PEN J.

F	E	D	C	B	A	
		16 Farm 2 Steers 8 Kill 1 - OLIVE & blue 8 Kill 2 - OLIVE	8 Farm 3 Heifers 8 Farm 4 Heifers Kill 2 - WHITE	8 Farm 1 Steers 8 Farm 2 Steers Kill 1 - DARK BLUE	16 Farm 3Heifers 8 Kill 1 WHITE & orange 8 Kill 2 - Light BLUE	M

G	H	I	J	K	L
4 Farm 2 Steers 4 Farm 1 Steers 4 Farm 3 Heifers 4 Farm 4 Heifers Kill 1 - YELLOW	4 Farm 2 Steers 4 Farm 1 Steers 4 Farm 3 Heifers 4 Farm 4 Heifers Kill 2 - ORANGE	16 Farm 4 Heifers 8 Kill 1 - WHITE & yellow 8 Kill 2 - PURPLE	8 Farm 1 Steers (Moved from PEN F) 8 Farm 2 Steers (Moved from PEN E) Kill 2 - GREEN	8 Farm 4 Heifers 8 Farm 3 Heifers Kill 1 - PINK	16 Farm 1 Steers 8 Kill 1 - OLIVE & red 8 Kill 2 - RED

Figure 10. Instruction sheet for transfer of cattle from trucks from 4 farms to vessel 2 pens

3.2.6 Phase 1 - Transport - Tasmania

Vessel 1 arrival and transfer to the abattoir

Vessel 1 docked at Devonport Wharf at 06:30am on the 23rd May with research personnel present to observe vessel unloading and to conduct a walk around inspection of each trailer to note any down cattle and general cattle demeanour. The two trailers were unloaded, connected to new prime movers and driven the 131km to the Abattoir.

Vessel 2 arrival, loading and transfer to the abattoir

Vessel 2 docked at Stanley Wharf at 06:00am on May 23rd where cattle were unloaded from the ship and loaded onto trucks to take them the 22 km to the abattoir. Vessel unloading and arrangements for local tray truck transfer to the abattoir were managed by the abattoir livestock manager. The research observer travelling on Vessel 2 provided detail of cattle grouping and ensured group segregation was maintained at all times. To comply with agreed curfew arrangements unloading began after 07.00am.

The research team recorded unloading times and followed the trucks to the abattoir. Figure 11 provides an overview of the unloading process.

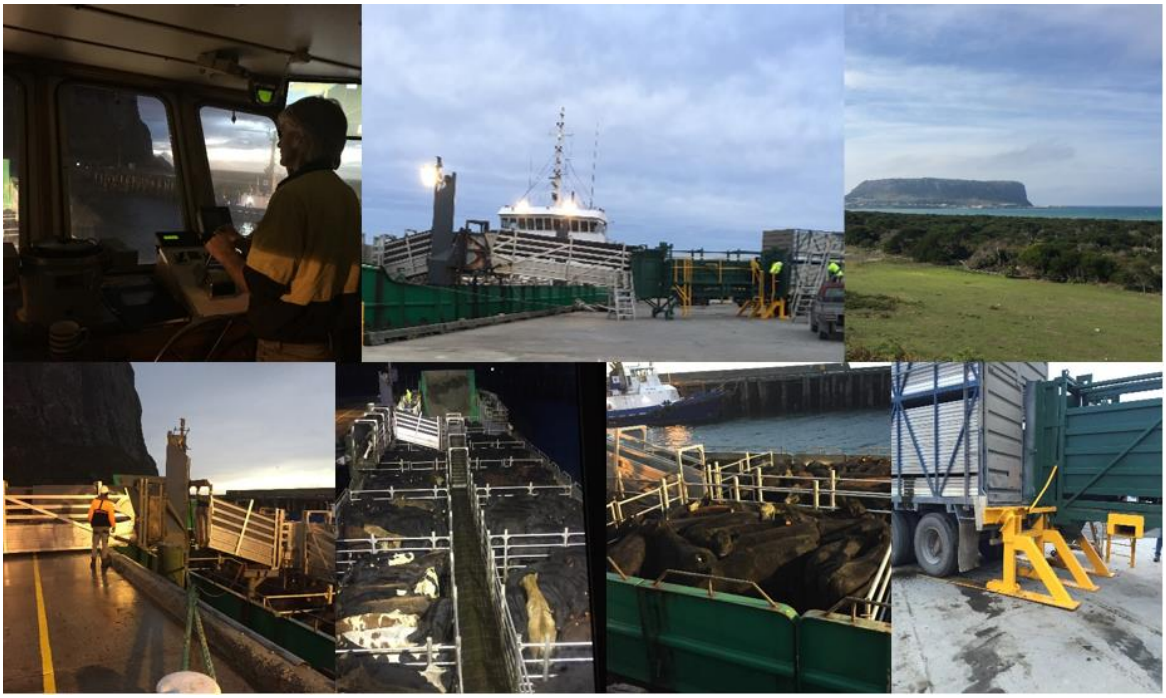


Figure 11: Vessel 2 unloading process

3.2.7 Phase 1 - Abattoir final drafting, lairage and holding paddock allocation

At the abattoir the research team supervised the unloading of trucks from both vessels and ensured group segregation was maintained in initial lairage penning. The running sheets provided to research and abattoir personnel for unloading and penning of the "green and orange" trucks from Vessel 1 are shown in Figure 12.

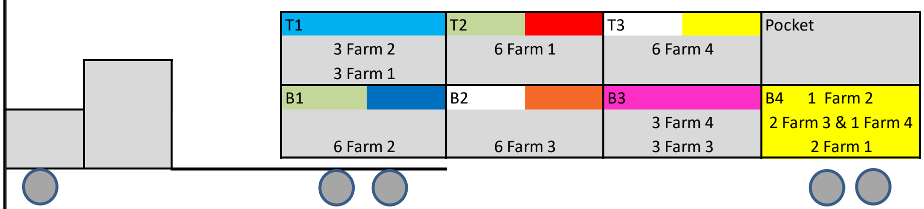
TRUCK PENNING ON VESSEL 1

Notes:
1. All cattle carry two coloured eartags, one in each ear.
2. Both eartags carry the same number which designates the source property.

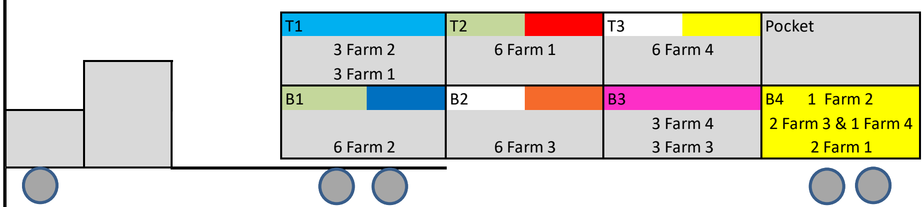
Eartag Nos	
1 to 61	FARM 1 STEERS
62 to 122	FARM 2 STEERS
123 to 183	FARM 3 HEIFERS
184 to 244	FARM 4 HEIFERS

3. The right eartags (Green on one trailer and Orange on the other - Purple tags are for Vessel 2) were used to designate the truck when drafting for loading.
4. The left eartag colour designates the treatment group.
5. Treatment groups **MUST** be kept together and not mixed during transport.
6. Some treatment groups (the 4 that have both a large cattle and small sheep tag in the left ear) **MUST** remain penned separately in lairage.
7. Other treatment groups can be mixed in lairage with left tags **OF THE SAME COLOUR** from both trucks (BLUE, PINK, YELLOW).
8. The penning for each of the two trucks from Vessel 1 is shown in the diagrams below.
9. The colours represent the LEFT EARTAG colour and treatment group.
10. Where there are two colours the left one is the Large cattle tag and the right a sheep tag that has been put in the cattle tag.
11. All cattle on the two Vessel 1 trailers are to be killed the first day (currently May 23rd).

"GREEN" TRUCK PENNING



"ORANGE" TRUCK PENNING



10. On arrival lairage penning required for trucks from Vessel 1 is as follows (Order as unloaded):

	Right Tag	Lge left TAG	Sheep Tag	Penning
GREEN TRUCK FROM VESSEL 1	GREEN	YELLOW		Box with other YELLOW from ORANGE truck and from VESSEL 2 (Will add to 28 Head)
	GREEN	PINK		Box with other PINK from ORANGE truck and from VESSEL 2 (Will add to 28 Head)
	GREEN	WHITE	ORANGE	Hold in own pen (6 Head)
	GREEN	OLIVE	BLUE	Hold in own pen (6 Head)
	GREEN	WHITE	YELLOW	Hold in own pen (6 Head)
	GREEN	OLIVE	RED	Hold in own pen (6 Head)
	GREEN	BLUE		Box with other BLUE from ORANGE truck and from VESSEL 2 (Will add to 28 Head)
ORANGE TRUCK FROM VESSEL 1	ORANGE	YELLOW		Box with other YELLOW from GREEN truck and from VESSEL 2 (Will add to 28 Head)
	ORANGE	PINK		Box with other PINK from GREEN truck and from VESSEL 2 (Will add to 28 Head)
	ORANGE	WHITE	ORANGE	Hold in own pen (6 Head)
	ORANGE	OLIVE	BLUE	Hold in own pen (6 Head)
	ORANGE	WHITE	YELLOW	Hold in own pen (6 Head)
	ORANGE	OLIVE	RED	Hold in own pen (6 Head)
	ORANGE	BLUE		Box with other BLUE from GREEN truck and from VESSEL 2 (Will add to 28 Head)

Figure 12. Running sheet provided for vessel 1 cattle transfer to lairage

Further running sheets as shown in Figure 13, 14 and 15 were provided to control transfer of cattle from Vessel 2 at the Stanley wharf to road transport and then after unloading to lairage pens and holding paddocks at the abattoir.

PENNING ON VESSEL 2

Notes:

1. All cattle carry two coloured eartags, one in each ear.
2. Both eartags carry the same number which designates the source property.

Eartag Nos	
1 to 61	Waverley Station steers
62 to 122	Arthur Stillmaker steers
123 to 183	Sustainable Agriculture heifers
184 to 244	Waverley Station heifers

3. All cattle from the STATESMAN will have large PURPLE right eartags.
4. The left eartag colour designates the treatment group.
5. Treatment groups MUST be kept together and not mixed during transport from Stanley to Smithton lairage.
6. Transport will therefore be required for 10 groups of 16 that need to be penned without mixing during transport from the vessel to Smithton.
6. On arrival at Smithton four pens will need to each be drafted into two further treatment groups requiring 4 separate lairage pens and 4 resting paddocks.
7. Other treatment groups can be mixed in lairage with left tags OF THE SAME COLOUR from the Mersey trucks (BLUE, PINK, YELLOW).
8. Four additional paddocks will needed for the GREEN, WHITE and ORANGE left eartag groups.
9. The STATESMAN penning is shown below.
10. The colours represent the LEFT EARTAG colour and treatment group.
11. Where there are two colours the left one is the Large cattle tag and the right a sheep tag that has been put in the cattle tag.
12. **80 cattle from the STATESMAN are to be killed the first day (currently May 23rd).**
13. **80 cattle from the STATESMAN are to be rested for 2 weeks and killed on June 6th.**

Figure 13. Penning on Vessel 2

F	E	D	C	B	A	
			8 Farm 3 Heifers	**8 Farm 1 Steers**		
		16 Farm 2 Steers			**16 Farm 3 Heifers**	
			8 Farm 4 Heifers	**8 Farm 2 Steers**		
		8 Kill 1 - OLIVE & blue			8 Kill 1 WHITE & orange	
		8 Kill 2 - OLIVE	Kill 2 - WHITE	Kill 1 - DARK BLUE	**8 Kill 2 - Light BLUE**	
						M
G	**H**	**I**	**J**	**K**	**L**	
4 Farm 2 Steers	**4 Farm 2 Steers**		**8 Farm 1 Steers**	**8 Farm 3 Heifers**		
4 Farm 1 Steers	**4 Farm 1 Steers**	**16 Farm 4 Heifers**			**16 Farm 1 Steers**	
4 Farm 3 Heifers	**4 Farm 3 Heifers**		**8 Farm 2 Steers**	**8 Farm 4 Heifers**		
4 Farm 4 Heifers	**4 Farm 4 Heifers**	8 Kill 1 - WHITE & yellow			8 Kill 1 - OLIVE & red	
Kill 1 - YELLOW	Kill 2 - ORANGE	**8 Kill 2 - PURPLE**	Kill 2 - GREEN	Kill 1 - PINK	**8 Kill 2 - RED**	

Figure 14. Abattoir lairage and holding paddock grouping for vessel 2 cattle groups

Right Tag	Vessel Pen	Drafting at Smithton	Large left Ear TAG	Small Sheep Tag	Penning in lairage for kill on Monday May 23	7 Resting Paddocks - for 2nd kill - June 6
PURPLE	C		WHITE			Single group of 16 in paddock for 14 days
PURPLE	D	**Have to be drafted**	OLIVE	BLUE	Single lairage pen of 8 head	
PURPLE	D	**after unloading**	OLIVE			Single group of 8 in paddock for 14 days
PURPLE	I	**Have to be drafted**	WHITE	YELLOW	Single lairage pen of 8 head	
PURPLE	I	**after unloading**	PURPLE			Single group of 8 in paddock for 14 days
PURPLE	J		GREEN			Single group of 16 in paddock for 14 days
PURPLE	K		PINK		Add to PINK from Vessel 1 - will make lairage pen up to 28	
PURPLE	B		BLUE		Add to BLUE from Vessel 1 - will make lairage pen up to 28	
PURPLE	A	**Have to be drafted**	WHITE	ORANGE	Single lairage pen of 8 head	
PURPLE	A	**after unloading**	LIGHT BLUE			Single group of 8 in paddock for 14 days
PURPLE	L	**Have to be drafted**	OLIVE	RED	Single lairage pen of 8 head	
PURPLE	L	**after unloading**	RED			Single group of 8 in paddock for 14 days
PURPLE	G		YELLOW		Add to YELLOW from Vessel 1 - will make lairage pen up to 28	
PURPLE	H		ORANGE			Single group of 16 in paddock for 14 days

Figure 15. Running sheets provided for Vessel 2 cattle transfer to lairage and holding paddocks

A final schematic diagram detailing the final lairage pen and paddock grouping for all the King Island cattle with number of head was provided as shown in Figure 16.

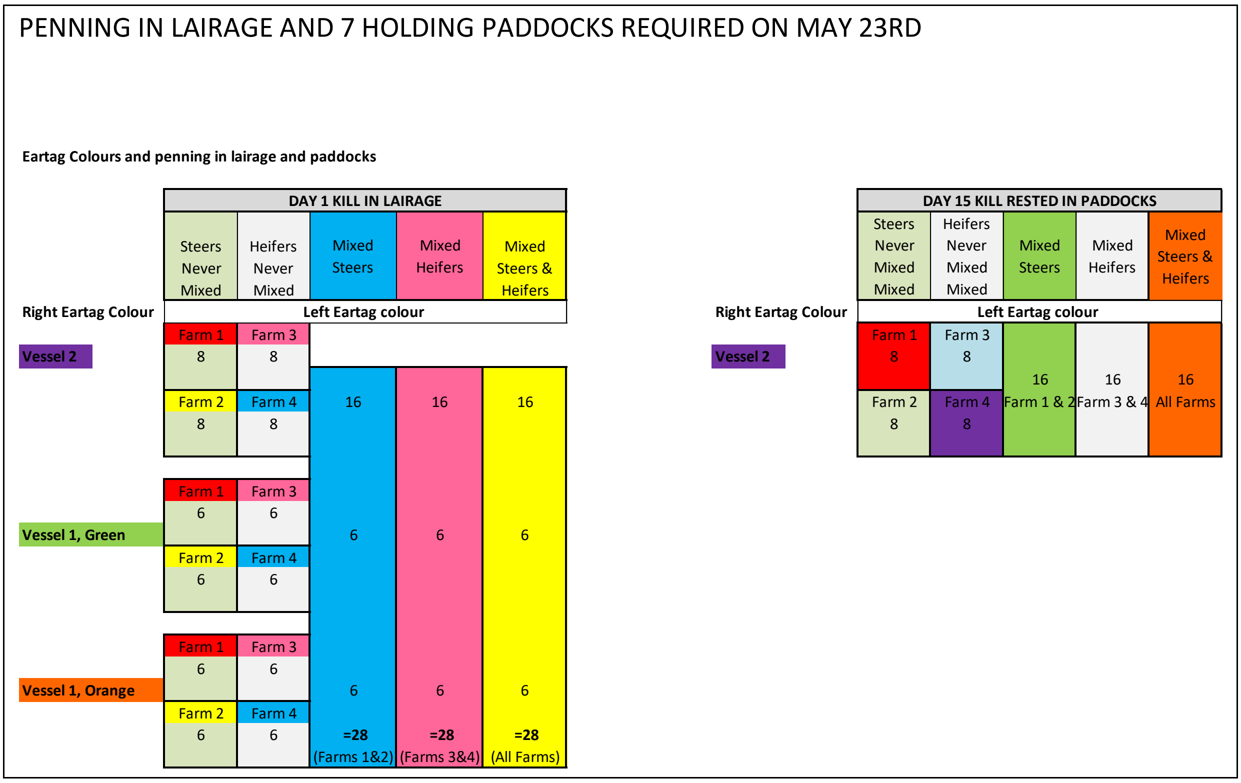


Figure 16. Representation of lairage and holding paddock allocation by eartag colour

Eighty cattle from vessel 2 were rested for 2 weeks in holding paddocks at the abattoir. The four never mixed groups from the source farms were maintained as separate groups with the mixed steer, heifer and sex groups in further paddocks as illustrated in Figure 16. Each paddock had a reasonable cover of feed with pasture samples collected by Murdoch University for testing. Supplemental grass silage was also provided over the holding period.

3.2.8 Phase 1 - Kill 1

A large research team from Polkinghornes, Sydney University, Melbourne University, Murdoch University, Joe Grose and MSA was required to fulfil all required research functions at the abattoir with significant additional assistance from abattoir management and staff. Prior to beginning the kill, abattoir engineering staff and research personnel mounted camera equipment to record cattle in the lead up race and knocking box as illustrated in Figure 17. The FLIR cameras were mounted above and beside the race leading to the knocking box and the field of view and function were checked. A further FLIR camera was mounted above the knocking box and 3 retinal cameras were mounted by engineering staff in the knocking box. Camera operation was monitored throughout the kill.

Figure 17. Recording at abattoir. Note FLIR cameras above and beside the lead up race and retinal cameras in knocking box

The 163 head (164 in the design less one rejected for loading at farm) designated for slaughter on arrival (May 23rd) were moved from lairage to slaughter in a designated order to ensure treatments were spread across the kill. For never mixed groups this was achieved by presenting the full pens of 6 from vessel 1 (orange and green trucks) or 8 from vessel 2 in the specified order.

Where sub groups were designated from the mixed pen groups any cattle could be drawn from the pen, in other words any 7 head from the total of 28 in the pen could be drawn to reduce overall disturbance. The kill order was discussed with abattoir management and communicated to lairage staff, supported by a laminated sheet as shown in Table 5. Lairage pen numbers were added to this sheet after the cattle had been penned.

Table 5. Designated kill order for May 23rd 2016 (First kill date)

	Left eartag colour		
	LARGE	small	
Kill Order	**No Head**		**Group**
1	6		(Never Mixed Heifers)
2	8		(Never Mixed Steers)
3	7		(Mixed Heifers & Steers)
4	8		(Never Mixed Heifers)
5	7		(Mixed Heifers (Only))
6	7		(Mixed Steers (Only))
7	6		(Never Mixed Steers)
8	8		(Never Mixed Heifers)
9	8		(Never Mixed Steers)
10	7		(Mixed Steers (Only))
11	7		(Mixed Heifers & Steers)
12	7		(Mixed Heifers (Only))
13	6		(Never Mixed Heifers)
14	7		(Mixed Steers (Only))
15	6		(Never Mixed Heifers)
16	7		(Mixed Heifers (Only))
17	6		(Never Mixed Steers)
18	7		(Mixed Heifers & Steers)
19	6		(Never Mixed Steers)
20	7		(Mixed Heifers (Only))
21	7		(Mixed Steers (Only))
22	7		(Mixed Heifers & Steers)
23	6		(Never Mixed Heifers)
24	6		(Never Mixed Steers)
	164		

The time of knocking each beast was recorded and eartag numbers were correlated to body number. Two vials of blood were collected at sticking then cooled and centrifuged. Data from the plant system provided a time at the scale and hot carcase weight immediately prior to chiller entry with a hot ossification score and hump height also recorded by Janine Lau, the senior MSA grader and research manager. Readings of loin pH & temperature were taken at hourly intervals by MSA staff for 5 hours post chiller entry.

A centrifuge was supplied by Murdoch University and installed in the plant laboratory. Immediately after the kill, the collected blood samples were centrifuged and the plasma and remaining material transferred to pre-labelled vials. Subsequently blood analysis was conducted at Murdoch University.

3.2.9 Phase 1 - Kill 1 grading, boning, cut collection and shipment

On the 24th May 2016, the left side of the 163 trial carcases were graded by MSA personnel. Full grade data was collected and DNA samples taken. In addition, research staff recorded HunterMeter and NIX colour readings from the graded loin of each carcase and a loin image was recorded with the Kuchida (MIJ) camera for analysis. Large brightly coloured 90 x 210mm laminated tags were pinned with 150mm stainless steel skewers to the oyster blade (FQ) and striploin (HQ) for identification during boning. The tags were consecutively numbered from 1 to 164 with both tags carrying the same number for each carcase together with a FQ or HQ notation.

The quarter ID tags were prepared prior to the kill together with five 50 x 65 coloured and laminated primal cut identification tags for each carcase as depicted in Figure 19 for carcases 1 to 3. The sides were marshalled for boning in a single run with abattoir management briefing the boning room staff on the required cutting lines for each of the 4 cuts to be collected – Striploin (STR045- HAM 2140), Tenderloin (TDR062 – HAM 2150), Oyster Blade (OYS036 – HAM 2303) and Outside (OUT005 – HAM 2030) and also directing and coordinating research personnel to ensure an effective collection.

The procedure to achieve accurate collection was developed by the boning room and abattoir managers in conjunction with Polkinghornes and MSA personnel prior to trial commencement. The tight boning room layout, simultaneous boning of fore and hind quarters, location of cut bagging and production speed required a detailed approach to ensure all 4 cuts could be collected with accurate ID retained.

As sides moved into the room for quartering and boning the ID tag on the striploin was moved up to the Outside Flat in the pre-trim area. Research personnel were positioned as directed at locations where cuts could be observed during boning and accessed post slicing. To ensure ID it was elected to bag cuts close to the slicing locations rather than risk ID loss as they moved by multiple conveyors to the standard bagging stations.

As the quarters moved down the boning chain the large carcase ID tags were observed and the number aligned with the small individual primal cut ID tags (see Figure 18). As a cut, for example striploin from carcase 1, was removed by the boner and trimmed by the slicer it was secured by research personnel who placed the corresponding primal tag on the cut surface and bagged the cut and tag. The carcase ID tags and pins were retrieved from the oyster blade and outside as bagged. To reduce complexity a full outside (HAM 2030) was collected with the outside flat (HAM 2050) and eye round (HAM 2040) separated at UNE during fabrication. The bagged and tagged cuts were then placed on the conveyor for vacuum packing. A count was made of stainless steel pins post collection to ensure all were retrieved.

49583	**49588**	**49593**
Carcass #1 AT L	**Carcass #2 AT L**	**Carcass #3 AT L**
TENDERLOIN (TDR062)	**TENDERLOIN (TDR062)**	**TENDERLOIN (TDR062)**
49584	**49589**	**49594**
Carcass #1 AT L	**Carcass #2 AT L**	**Carcass #3 AT L**
STRIPLOIN (STR045)	**STRIPLOIN (STR045)**	**STRIPLOIN (STR045)**
49585	**49590**	**49595**
Carcass #1 AT L	**Carcass #2 AT L**	**Carcass #3 AT L**
OYSTER BLADE (OYS036)	**OYSTER BLADE (OYS036)**	**OYSTER BLADE (OYS036)**
49586	**49591**	**49596**
Carcass #1 AT L	**Carcass #2 AT L**	**Carcass #3 AT L**
OUTSIDE	**OUTSIDE**	**OUTSIDE**
49587	**49592**	**49597**
Carcass #1 AT L	**Carcass #2 AT L**	**Carcass #3 AT L**
EYE ROUND (EYE075)	**EYE ROUND (EYE075)**	**EYE ROUND (EYE075)**

Figure 18. Example primal tags used for identification from boning

Cuts with research primal tags were identified by abattoir staff at the vacuum packing stations, checked for adequate seal and any leakers re-bagged. They were then placed in cartons, weighed and identified with a special code to ensure the shipment could be identified from general commercial product. Cartons of trial product were then chilled, palletised and loaded in a shipping container for transport to the Melbourne distribution centre.

The product was collected by Polkinghornes from the distribution centre and transported with a hired refrigerated truck to the University of New England meat laboratory for further fabrication to consumer sensory and objective samples.

3.2.10 Phase 1 - Kill 2

The 80 cattle that had been rested for 2 weeks were moved from paddocks to lairage pens retaining their individual groups for slaughter on June 6th 2016. Prior to the kill, research and abattoir personnel again mounted FLIR and retinal cameras as for the first kill. Lairage penning reflected the previous 14 days paddock grouping to maintain treatment groups as displayed in Table 6.

Table 6. Paddock and lairage segregation of Phase 1 cattle rested for 2 weeks prior to slaughter

DAY 15 KILL RESTED IN PADDOCKS				
Steers Never Mixed	Heifers Never Mixed	Mixed Steers	Mixed Heifers	Mixed Steers & Heifers
Mixed	Mixed			
Left Eartag Colour				
Farm 1 8	Farm 3 8	16 Farm 1 & 2	16 Farm 3 & 4	16 All Farms
Farm 2 8	Farm 4 8			

The cattle were moved to slaughter in a designated order with 10 groups of 8 head as displayed in Table 7 to ensure treatments (defined by LEFT ear tag colour) were distributed across the kill.

Table 7. Treatment kill order for second slaughter

Left Eartag Colour			
Kill Order	**No Head**	**Group**	
1	8	(Mixed Heifers & Steers)	
2	8	(Mixed Heifers (Only))	
3	8	(Never Mixed Steers)	
4	8	(Mixed Steers (Only))	
5	8	(Never Mixed Heifers)	
6	8	(Never Mixed Steers)	
7	8	(Mixed Heifers & Steers)	
8	8	(Never Mixed Heifers)	
9	8	(Mixed Heifers (Only))	
10	8	(Mixed Steers (Only))	
	80		

Operating procedures for FLIR and retinal camera recording, animal to carcase ID linkage, blood collection, pH and temperature decline were as detailed for the first kill.

On 7th June 2016 all trial carcases were MSA graded, identified with research carcase labels and cuts from each left side collected and identified at boning as per kill 1 prior to shipment by hired refrigerated transport to University of New England, Armidale for further sample fabrication.

4 Methodology – Phase 2 Collection

4.1 Trial Design - Phase 2

Consistent with the Phase 1 King Island trial design, Phase 2 was designed to induce stress through mixing and transport, to measure the stress impact on eating quality as determined by consumers and to attempt to remediate the induced stress through rest with the ensuing eating quality also determined through consumer testing as per the Standard MSA protocols (Gee et al. 2005). Linkage between the two phases was aided by use of a common abattoir.

The design specified 240 cattle from 4 North Western Tasmanian farms each supplying 60 cattle, with two farms supplying steers and 2 farms supplying heifers. The 60 head per farm design was arrived at after power analysis to assess adequate sample size in conjunction with truck pen size to ensure transport within appropriate welfare guidelines and to enable balanced numbers within treatments.

Treatments were devised to create variable stress impact through mixing of cattle across farms and within and across sexes with control groups of steers and heifers that were never mixed. Each of the mixing treatment groups was divided into three subsets with each subset subjected to one of the three transport pathways which comprised by truck from farm to abattoir via Saleyard A located in North Eastern Tasmania, by truck from farm to abattoir via Saleyard B located in North Western Tasmania or by truck from farm direct to the abattoir. On arrival at the abattoir a sub set of each treatment group from each farm were to be killed on arrival day as per normal practice and the remainder rested on good pasture for 2 weeks prior to slaughter. Table 8 displays the allocation numbers by farm and treatment.

Table 8. Number of head per farm and allocation by treatment

	DIRECT TO SLAUGHTER				SALEYARD A						SALEYARD B						
	Steers		Heifers		MIXED STEERS		MIXED HEIFERS		MIXED SEX		MIXED STEERS		MIXED HEIFERS		MIXED SEX		REPLICATE
	Kill day 1	Kill day 14	Kill day 1	Kill day 14	Kill day 1	Kill day 14	Kill day 1	Kill day 14	Kill day 1	Kill day 14	Kill day 1	Kill day 14	Kill day 1	Kill day 14	Kill day 1	Kill day 14	TOTAL
Farm 1	12	12			6	6			3	3	6	6			3	3	60
Farm 2	12	12			6	6			3	3	6	6			3	3	60
Farm 3			12	12			6	6	3	3			6	6	3	3	60
Farm 4			12	12			6	6	3	3			6	6	3	3	60
TREATMENT TOTALS	24	24	24	24	12	12	12	12	12	12	12	12	12	12	12	12	240
	RECOVERY TEST		Kill day 1	Kill day 14													
	18		30	30													
	18		30	30													
	18		30	30													
	18		30	30													
	72		120	120													

As in Phase 1, eartag colours were to be assigned to identify farm of origin, treatment groups and kill date. The research design required cattle to be sequentially drafted to treatment with surplus cattle drafted off during the initial handling. Also as in Phase 1, a combination of left and right eartag colours were designated to visually indicate the treatment group, destination and truck loading.

While the treatments were identical, the research design differed from Phase 1 in that the never mixed groups were to be trucked direct to slaughter on the day before slaughter for both kills and on different transport to the saleyard groups. All cattle allocated to the saleyards were to be mixed at the yards either within same sex or mixed sex groups from other farms. All saleyard cattle were to be trucked on a common day with equal numbers, 36 head from each farm, loaded and 18 from each farm unloaded at each of the two saleyards.

To maintain a common time from farm to slaughter loading at the four farms had to be conducted at similar times requiring 3 teams to be designated and each provided with clear detailed instruction of drafting and loading detail. Similar instruction regarding unloading and mixing of cattle was designated for saleyard staff and research coordinators at each location.

To accommodate the sale days being a Tuesday at saleyard A and Wednesday at saleyard B, the saleyard groups were to be loaded mid Monday afternoon with the saleyard B group held in covered dirt pens and fed hay for the day prior to the sale whereas the saleyard A group were to be included in sale penning on arrival and held on hay the following day. Mixing treatments were to be applied on arrival at the saleyard with cattle from two or all four farms boxed into common pens as designated by eartag colour. The research protocol required the saleyard groups to be handled as would occur during a normal sale. This required them to be drafted into pseudo sale lots, weighed and paint branded then penned in sale groups without mixing their treatment groups. During the sale each group was to be penned within actual sale lots to ensure all sale day activity included the trial cattle.

Cattle from saleyards A and B were to be transported to the abattoir late on the Wednesday evening as were the first kill direct from farm cattle. On arrival at the abattoir a designated half of each saleyard delivery, comprising equal numbers from each farm and treatment within farm, were to be held in lairage for kill the following morning whereas the other half were to be rested in paddocks for two weeks prior to the second kill. The direct consignment cattle for the second kill were to be trucked the day prior.

Kill procedures, recording, grading and cut collection procedures were designated to be identical to Phase 1. A detailed timetable for Phase 2 may be found at Attachment D.

4.2 Materials and Methods – Phase 2

4.2.1 Phase 2 - Scoping visit

A scoping visit was required to ensure that multiple groups of cattle could be processed on farm, be transported in their specific groups from the farm to the two saleyards used in the trial, that they could be held there in their groups until sale and then transported to slaughter or holding paddocks, all while still retaining their specific groups. Information gathered on the scoping visit informed modifications made to the materials and methods in order to ensure the integrity of the trial design while meeting practical considerations.

Following prior discussions the collaborating abattoir had identified four properties that were suitable for the proposed trial and were willing to participate. Farms were required to have sufficient numbers of single sex cattle such that they could supply 60 head of one sex on one day that would meet the abattoir specifications. It was required that the farms have reasonable standard yards with scales and the capacity to draft a minimum of 3 ways on the loading day, with additional draft capacity an advantage. A suitable loading ramp and all weather access for single 40 ft trailer double deck trucks was required. The properties were located at Mella, Marrawah, North Motton and Arthur River in North Western Tasmania.

The scoping visit to both saleyards took place on the 25th May to view the physical facilities and discuss the trial requirements. The farms were visited on Sunday June 5th to ascertain the layout of each farm, available yards, review transport arrangements and brief the participants to ensure their understanding of the level of detail and accuracy required in all activities in the trial.

This visit coincided with an extreme weather event involving heavy rain, extreme cold and local flooding.

As in Phase 1 the unique layout of each set of yards on the 4 farms informed the list of resources required for each farm visit taking into account factors such as:

- Cattle yards with no electricity available for the required equipment which necessitated generators and an assessment of how many extension leads and how long they needed to be to reach all the equipment required on each subsequent farm visit.
- The need to determine the best location and possible methods of securing the camera equipment in order to ensure the correct view and angle required to take the measurements of each beast as it went up the race, was held in the crush and as it exited from the crush.
- The individual cattle yard details were photographed to review the most appropriate method of mounting the cameras which would in turn inform the list of resources required for each subsequent farm visit.
- The yards' capacity to safely draft the cattle into the required lots to meet the penning design.
- Wet weather contingency options.

Saleyards observations and discussion confirmed that the cattle could be unloaded and held until sale with adequate feed and water in the correct penning arrangements and maintained in the allocated groups for transport to the abattoir after the sale.

Abattoir arrangements were confirmed to be the same as for Phase 1. The research team was also the same as in Phase 1.

Recording sheets and eartags were prepared prior to travelling to Tasmania for field operations.

4.2.2 Phase 2 - Cattle selection, identification and baseline measurements

On the 7th, 8th and 9th June 2016, a project team travelled to each farm to select and tag 60 cattle for the trial, assign them to groups and then gather the baseline measurements required. These visits were coordinated with the second Phase 1 kill and cut collection and adapted to take advantage of brief respites in the prevailing extremely wet and cold weather.

The team comprised representatives from Polkinghornes, MSA, Murdoch University, Melbourne University, Sydney University, who visited Farm 1 and Farm 2 on the June 8th, followed by Farm 3 and Farm 4 on the June 9th.

The yards were set up with:

- An FLIR camera mounted above the race with mounting point and method adapted to suit each individual layout and provide a continuous clear view of cattle moving up the race.
- An FLIR camera mounted on a tripod just opposite and at right angles to the crush to enable a continuous picture to be taken of each animals head and eye while the head was held in the crush.
- Two flight speed sensors mounted at suitable positions after the crush in a position where they would not be damaged by the cattle. The position depended on the unique layout of each set of yards. The closest sensor to the crush was positioned more than 1 metre away to avoid breaking the beam during eartag application and retinal scanning. The distance between the two sensors was measured as individual yard layouts dictated the possible locations.
- Where necessary a portable generator was located at a distance from the yards to reduce noise and extension leads run as required.
- The property scales were checked for function with batteries attached where needed.
- A suitable area was located to lay out eartags, applicators and to record data.
- Access and position for the retinal camera operator was identified.

The mob of cattle was mustered quietly to the yards by farm personnel and then rested for approximately one hour. All cattle were then processed through the race and crush and any animal that did not meet the buyers specifications, was not more or less uniform or that was much heavier or lighter than the rest of the mob was drafted off and not used for the trial. The first 60 animals that met the buyer's specifications were selected and tagged sequentially in the order of the pre-arranged ear tags.

Eartag colour was utilised to identify the treatment group, farm and initial trucking destination and to facilitate drafting immediately prior to transport. The right ear tag colour corresponded to the destination saleyard or control group. The left ear tag identified the treatment group and the farm. To generate sufficient unique colour combinations that would be clearly visible while safely drafting cattle, and address a previously noted tendency for pink eartags to fade toward white, tags allocated to the mixed heifer groups comprised a cattle tag of one colour with a different coloured sheep eartag punched into it so that there were two colours in that ear.

The allocation of cattle to treatment within farm and associated eartagging is shown in Table 9. The detailed individual sheets used to control the sequential tagging are displayed in Table 10.

Table 9. Allocation of cattle to treatment, saleyard and kill for Phase 2 saleyard cattle

Farm	Eartags	Treatment	Kill	No Head	Left Eartag	Control/Saleyard	Right Eartag
1	245 to 304	Never Mixed Steers	1	12	RED	Control - direct from farm to kill	WHITE
		Never Mixed Steers	2	12	RED	Control - direct from farm to kill	ORANGE
		Mixed Steers	1	6	BLUE	A	YELLOW
		Mixed Steers	1	6	BLUE	B	GREEN
		Mixed Steers	2	6	GREEN	A	YELLOW
		Mixed Steers	2	6	GREEN	B	GREEN
		Mixed Sex	1	3	YELLOW	A	YELLOW
		Mixed Sex	1	3	YELLOW	B	GREEN
		Mixed Sex	2	3	ORANGE	A	YELLOW
		Mixed Sex	2	3	ORANGE	B	GREEN
2	305 to 364	Never Mixed Steers	1	12	OLIVE	Control - direct from farm to kill	WHITE
		Never Mixed Steers	2	12	OLIVE	Control - direct from farm to kill	ORANGE
		Mixed Steers	1	6	BLUE	A	YELLOW
		Mixed Steers	1	6	BLUE	B	GREEN
		Mixed Steers	2	6	GREEN	A	YELLOW
		Mixed Steers	2	6	GREEN	B	GREEN
		Mixed Sex	1	3	YELLOW	A	YELLOW
		Mixed Sex	1	3	YELLOW	B	GREEN
		Mixed Sex	2	3	ORANGE	A	YELLOW
		Mixed Sex	2	3	ORANGE	B	GREEN
3	365 to 424	Never Mixed Heifers	1	12	Light BLUE	Control - direct from farm to kill	WHITE
		Never Mixed Heifers	2	12	Light BLUE	Control - direct from farm to kill	ORANGE
		Mixed Heifers	1	6	PINK w red	A	YELLOW
		Mixed Heifers	1	6	PINK w red	B	GREEN
		Mixed Heifers	2	6	WHITE w blue	A	YELLOW
		Mixed Heifers	2	6	WHITE w blue	B	GREEN
		Mixed Sex	1	3	YELLOW	A	YELLOW
		Mixed Sex	1	3	YELLOW	B	GREEN
		Mixed Sex	2	3	ORANGE	A	YELLOW
		Mixed Sex	2	3	ORANGE	B	GREEN
4	425 to 484	Never Mixed Heifers	1	12	PURPLE	Control - direct from farm to kill	WHITE
		Never Mixed Heifers	2	12	PURPLE	Control - direct from farm to kill	ORANGE
		Mixed Heifers	1	6	PINK w red	A	YELLOW
		Mixed Heifers	1	6	PINK w red	B	GREEN
		Mixed Heifers	2	6	WHITE w blue	A	YELLOW
		Mixed Heifers	2	6	WHITE w blue	B	GREEN
		Mixed Sex	1	3	YELLOW	A	YELLOW
		Mixed Sex	1	3	YELLOW	B	GREEN
		Mixed Sex	2	3	ORANGE	A	YELLOW
		Mixed Sex	2	3	ORANGE	B	GREEN

Table 10. Tagging sequence for each Phase 2 property

		FARM 1				FARM 2	
Right Eartag Colour			Left Eartag Colour	Right Eartag Colour			Left Eartag Colour
Eartag	Kill	Group	Treatment	Eartag	Kill	Group	Treatment
245	K1	Control	Steer	305	K1	Control	Steer
246	K2	Saleyard B	Mixed Steers	306	K2	Saleyard B	Mixed Steers
247	K1	Saleyard A	Mixed Steers	307	K1	Saleyard A	Mixed Steers
248	K2	Control	Steer	308	K2	Control	Steer
249	K1	Saleyard B	Mixed Steers	309	K1	Saleyard B	Mixed Steers
250	K2	Saleyard A	Mixed Steers	310	K2	Saleyard A	Mixed Steers
251	K1	Control	Steer	311	K1	Control	Steer
252	K2	Saleyard B	Mixed Steers & Heifers	312	K2	Saleyard B	Mixed Steers & Heifers
253	K1	Saleyard A	Mixed Steers & Heifers	313	K1	Saleyard A	Mixed Steers & Heifers
254	K2	Control	Steer	314	K2	Control	Steer
255	K1	Control	Steer	315	K1	Control	Steer
256	K2	Saleyard A	Mixed Steers & Heifers	316	K2	Saleyard A	Mixed Steers & Heifers
257	K1	Saleyard B	Mixed Steers & Heifers	317	K1	Saleyard B	Mixed Steers & Heifers
258	K2	Control	Steer	318	K2	Control	Steer
259	K1	Saleyard A	Mixed Steers	319	K1	Saleyard A	Mixed Steers
260	K2	Saleyard B	Mixed Steers	320	K2	Saleyard B	Mixed Steers
261	K1	Control	Steer	321	K1	Control	Steer
262	K2	Saleyard A	Mixed Steers	322	K2	Saleyard A	Mixed Steers
263	K1	Saleyard B	Mixed Steers	323	K1	Saleyard B	Mixed Steers
264	K2	Control	Steer	324	K2	Control	Steer
265	K1	Control	Steer	325	K1	Control	Steer
266	K2	Saleyard A	Mixed Steers	326	K2	Saleyard A	Mixed Steers
267	K1	Saleyard B	Mixed Steers	327	K1	Saleyard B	Mixed Steers
268	K2	Control	Steer	328	K2	Control	Steer
269	K1	Saleyard A	Mixed Steers	329	K1	Saleyard A	Mixed Steers
270	K2	Saleyard B	Mixed Steers	330	K2	Saleyard B	Mixed Steers
271	K1	Control	Steer	331	K1	Control	Steer
272	K2	Saleyard A	Mixed Steers & Heifers	332	K2	Saleyard A	Mixed Steers & Heifers
273	K1	Saleyard B	Mixed Steers & Heifers	333	K1	Saleyard B	Mixed Steers & Heifers
274	K2	Control	Steer	334	K2	Control	Steer
275	K1	Control	Steer	335	K1	Control	Steer
276	K2	Saleyard B	Mixed Steers & Heifers	336	K2	Saleyard B	Mixed Steers & Heifers
277	K1	Saleyard A	Mixed Steers & Heifers	337	K1	Saleyard A	Mixed Steers & Heifers
278	K2	Control	Steer	338	K2	Control	Steer
279	K1	Saleyard B	Mixed Steers	339	K1	Saleyard B	Mixed Steers
280	K2	Saleyard A	Mixed Steers	340	K2	Saleyard A	Mixed Steers
281	K1	Control	Steer	341	K1	Control	Steer
282	K2	Saleyard B	Mixed Steers	342	K2	Saleyard B	Mixed Steers
283	K1	Saleyard A	Mixed Steers	343	K1	Saleyard A	Mixed Steers
284	K2	Control	Steer	344	K2	Control	Steer
285	K1	Control	Steer	345	K1	Control	Steer
286	K2	Saleyard B	Mixed Steers	346	K2	Saleyard B	Mixed Steers
287	K1	Saleyard A	Mixed Steers	347	K1	Saleyard A	Mixed Steers
288	K2	Control	Steer	348	K2	Control	Steer
289	K1	Saleyard B	Mixed Steers	349	K1	Saleyard B	Mixed Steers
290	K2	Saleyard A	Mixed Steers	350	K2	Saleyard A	Mixed Steers
291	K1	Control	Steer	351	K1	Control	Steer
292	K2	Saleyard B	Mixed Steers & Heifers	352	K2	Saleyard B	Mixed Steers & Heifers
293	K1	Saleyard A	Mixed Steers & Heifers	353	K1	Saleyard A	Mixed Steers & Heifers
294	K2	Control	Steer	354	K2	Control	Steer
295	K1	Control	Steer	355	K1	Control	Steer
296	K2	Saleyard A	Mixed Steers & Heifers	356	K2	Saleyard A	Mixed Steers & Heifers
297	K1	Saleyard B	Mixed Steers & Heifers	357	K1	Saleyard B	Mixed Steers & Heifers
298	K2	Control	Steer	358	K2	Control	Steer
299	K1	Saleyard A	Mixed Steers	359	K1	Saleyard A	Mixed Steers
300	K2	Saleyard B	Mixed Steers	360	K2	Saleyard B	Mixed Steers
301	K1	Control	Steer	361	K1	Control	Steer
302	K2	Saleyard A	Mixed Steers	362	K2	Saleyard A	Mixed Steers
303	K1	Saleyard B	Mixed Steers	363	K1	Saleyard B	Mixed Steers
304	K2	Control	Steer	364	K2	Control	Steer

		FARM 3					FARM 4	
Right Eartag Colour			Left Eartag Colour		Right Eartag Colour			Left Eartag Colour
Eartag	Kill	Group	Treatment		Eartag	Kill	Group	Treatment
365	K1	Control	Heifer		425	K1	Control	Heifer
366	K2	Saleyard B	Mixed Heifers		426	K2	Saleyard B	Mixed Heifers
367	K1	Saleyard A	Mixed Heifers		427	K1	Saleyard A	Mixed Heifers
368	K2	Control	Heifer		428	K2	Control	Heifer
369	K1	Saleyard B	Mixed Heifers		429	K1	Saleyard B	Mixed Heifers
370	K2	Saleyard A	Mixed Heifers		430	K2	Saleyard A	Mixed Heifers
371	K1	Control	Heifer		431	K1	Control	Heifer
372	K2	Saleyard B	Mixed Steers & Heifers		432	K2	Saleyard B	Mixed Steers & Heifers
373	K1	Saleyard A	Mixed Steers & Heifers		433	K1	Saleyard A	Mixed Steers & Heifers
374	K2	Control	Heifer		434	K2	Control	Heifer
375	K1	Control	Heifer		435	K1	Control	Heifer
376	K2	Saleyard A	Mixed Steers & Heifers		436	K2	Saleyard A	Mixed Steers & Heifers
377	K1	Saleyard B	Mixed Steers & Heifers		437	K1	Saleyard B	Mixed Steers & Heifers
378	K2	Control	Heifer		438	K2	Control	Heifer
379	K1	Saleyard A	Mixed Heifers		439	K1	Saleyard A	Mixed Heifers
380	K2	Saleyard B	Mixed Heifers		440	K2	Saleyard B	Mixed Heifers
381	K1	Control	Heifer		441	K1	Control	Heifer
382	K2	Saleyard A	Mixed Heifers		442	K2	Saleyard A	Mixed Heifers
383	K1	Saleyard B	Mixed Heifers		443	K1	Saleyard B	Mixed Heifers
384	K2	Control	Heifer		444	K2	Control	Heifer
385	K1	Control	Heifer		445	K1	Control	Heifer
386	K2	Saleyard A	Mixed Heifers		446	K2	Saleyard A	Mixed Heifers
387	K1	Saleyard B	Mixed Heifers		447	K1	Saleyard B	Mixed Heifers
388	K2	Control	Heifer		448	K2	Control	Heifer
389	K1	Saleyard A	Mixed Heifers		449	K1	Saleyard A	Mixed Heifers
390	K2	Saleyard B	Mixed Heifers		450	K2	Saleyard B	Mixed Heifers
391	K1	Control	Heifer		451	K1	Control	Heifer
392	K2	Saleyard A	Mixed Steers & Heifers		452	K2	Saleyard A	Mixed Steers & Heifers
393	K1	Saleyard B	Mixed Steers & Heifers		453	K1	Saleyard B	Mixed Steers & Heifers
394	K2	Control	Heifer		454	K2	Control	Heifer
395	K1	Control	Heifer		455	K1	Control	Heifer
396	K2	Saleyard B	Mixed Steers & Heifers		456	K2	Saleyard B	Mixed Steers & Heifers
397	K1	Saleyard A	Mixed Steers & Heifers		457	K1	Saleyard A	Mixed Steers & Heifers
398	K2	Control	Heifer		458	K2	Control	Heifer
399	K1	Saleyard B	Mixed Heifers		459	K1	Saleyard B	Mixed Heifers
400	K2	Saleyard A	Mixed Heifers		460	K2	Saleyard A	Mixed Heifers
401	K1	Control	Heifer		461	K1	Control	Heifer
402	K2	Saleyard B	Mixed Heifers		462	K2	Saleyard B	Mixed Heifers
403	K1	Saleyard A	Mixed Heifers		463	K1	Saleyard A	Mixed Heifers
404	K2	Control	Heifer		464	K2	Control	Heifer
405	K1	Control	Heifer		465	K1	Control	Heifer
406	K2	Saleyard B	Mixed Heifers		466	K2	Saleyard B	Mixed Heifers
407	K1	Saleyard A	Mixed Heifers		467	K1	Saleyard A	Mixed Heifers
408	K2	Control	Heifer		468	K2	Control	Heifer
409	K1	Saleyard B	Mixed Heifers		469	K1	Saleyard B	Mixed Heifers
410	K2	Saleyard A	Mixed Heifers		470	K2	Saleyard A	Mixed Heifers
411	K1	Control	Heifer		471	K1	Control	Heifer
412	K2	Saleyard B	Mixed Steers & Heifers		472	K2	Saleyard B	Mixed Steers & Heifers
413	K1	Saleyard A	Mixed Steers & Heifers		473	K1	Saleyard A	Mixed Steers & Heifers
414	K2	Control	Heifer		474	K2	Control	Heifer
415	K1	Control	Heifer		475	K1	Control	Heifer
416	K2	Saleyard A	Mixed Steers & Heifers		476	K2	Saleyard A	Mixed Steers & Heifers
417	K1	Saleyard B	Mixed Steers & Heifers		477	K1	Saleyard B	Mixed Steers & Heifers
418	K2	Control	Heifer		478	K2	Control	Heifer
419	K1	Saleyard A	Mixed Heifers		479	K1	Saleyard A	Mixed Heifers
420	K2	Saleyard B	Mixed Heifers		480	K2	Saleyard B	Mixed Heifers
421	K1	Control	Heifer		481	K1	Control	Heifer
422	K2	Saleyard A	Mixed Heifers		482	K2	Saleyard A	Mixed Heifers
423	K1	Saleyard B	Mixed Heifers		483	K1	Saleyard B	Mixed Heifers
424	K2	Control	Heifer		484	K2	Control	Heifer

FLIR thermal images were obtained of each animal moving through the race to the crush. Should the animal meet specifications, then the animal's new eartag number was recorded. Should the animal be rejected then it was also noted within order to align FLIR output with animal ID.

For each selected animal

- A crush score (1 to 5 scale) was recorded after observation of behaviour in the crush during weighing.
- Liveweight and any existing farm tags were recorded.
- A hand held FLIR camera was used to obtain retinal images for selected cattle when caught in the crush head bail.
- A minimum of 10 seconds of video was recorded with a hand held retinal camera.
- Any existing farm eartags were removed to avoid any later confusion and two trial eartags applied in accordance with sequential allocation to treatment groups.
- A second FLIR and retinal reading was taken after the eartagging interaction.
- As the animal was let out of the crush the flight speed was recorded.
- The selected animals were returned to their designated paddock as a single mob of 60 head

4.2.3 Phase 2 - Transport to saleyards

Running sheets were prepared for each farm to specify drafting and recording details with an example displayed in Figure 19.

The trial mobs were mustered by farm personnel on the afternoon of June 27th 2016, two weeks after cattle selection, eartagging and baseline measurements were taken. One animal was rejected due to exhibiting extreme stress during mustering. The project team prepared the yards as per the first visit. The cameras were mounted as before but flight speed was not recorded due to not having sufficient units to manage all sites. The timing of the two sales and associated logistics effectively required cattle from each farm to be loaded simultaneously, requiring a team of people at each of the 4 farms. The teams comprised representatives from Polkinghornes, MSA, Murdoch University, Melbourne University, Sydney University in addition to the farm personnel. Each team:

- Recorded FLIR thermographic images in the race leading to crush.
- Obtained retinal readings for a minimum of 10 seconds for all cattle in the crush head bail.
- Recorded live weight.
- Replaced any missing ear tags
- Cattle were drafted according to the designated saleyard indicated by right eartag colour
- Control cattle were returned to nearby paddocks.
- Prior to loading each saleyard group was further drafted on left eartag colour for truck penning.
- Supervised drafting and truck loading.

FARM 2

Loading is 6 per pen based on eartag colour.

Drafting and loading detail are as follows:

1. Cattle working prior to loading.

- Attach camera above race and connect to battery power.
- Mount scales in crush.
- Muster 60 head to yards.
- Run all cattle through race to crush.
- Record weight and eartag number in order of presentation and mark off from eartag order list.
- Replace any missing eartags (Use laminated master list to reference colours)
- Catch head and take retinal video for 10 seconds.
- Record eye reading and number.
- Attach accelerometer to right ear tag of selected cattle and record number.

2. Draft to groups on RIGHT ear tag colour.

Drafting to be done as decided by Farm representative either direct from the crush, post weighing or a combination to suit yards.

GROUP 1. 24 head - 12 with WHITE RIGHT ear tags and 12 with ORANGE RIGHT ear tags.
These groups can be returned to the paddock.
The WHITE tags will be trucked to Abattoir on Wednesday June 29th.
The ORANGE tags will be trucked to Abattoir on Wednesday July 13th.

GROUP 2. 18 head with YELLOW RIGHT ear tags - to be loaded for Saleyard A.

GROUP 3. 18 head - with GREEN RIGHT ear tags - to be loaded to Saleyard B.

3. Draft for truck pen loading based on LEFT ear tag colour.

For the Saleayrd A cattle (18 head with **YELLOW RIGHT** ear tags)
GROUP 1. 6 head with **BLUE LEFT** ear tags.
GROUP 2. 6 head with **GREEN LEFT** ear tags.
GROUP 3. 6 remaining - 3 will have **YELLOW** and 3 will have **ORANGE** LEFT eartags.

For the Saleyard B cattle (18 head with **GREEN RIGHT** ear tags)
GROUP 4. 6 head with **BLUE LEFT** ear tags.
GROUP 5. 6 head with **GREEN LEFT** ear tags.
GROUP 6. 6 remaining - 3 will have **YELLOW** and 3 will have **ORANGE** LEFT eartags.

The GROUP numbers above represent the loading order. Farm representative to control drafting timing and sequence to suit yard workings.

Figure 19. Running sheet with detail of required drafting prior to transport

Four trucks were used with each truck visiting only one farm to collect the trial cattle allocated to the saleyard treatments. Four pens per deck double deck trailers were used for all bar one farm where a 3 pen per deck trailer was used due to the heavier animal weight. The trucks travelled from each farm to Saleyard B, unloaded the designated cattle then proceeded to Saleyard A. 36 head from each property were loaded at approximately 4pm to achieve a constant time from property to slaughter. The truck penning (6 pens of 6 from each property) was designated by eartag colours.

The required truck loading sequence was shown in laminated instruction sheets given to each farm team and truck driver with an example displayed in Figure 20. The colours shown at the top of each pen are those corresponding to the LEFT eartags.

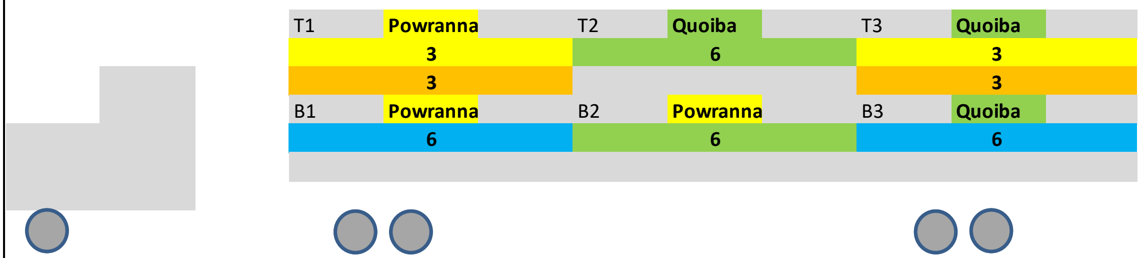
Twelve of the 24 head that remained on each farm were loaded on the afternoon of June 29th and transported to the abattoir, arriving at a similar time to the saleyard groups. The final 12 head were trucked to the abattoir late in the afternoon of July 13th to provide a direct delivery control to the saleyard groups that had been rested for 14 days.

LOADING START TIME (Truck at ramp)

2. Loading sequence

Load GROUP 1 - 6 head with **YELLOW RIGHT** and **BLUE LEFT** ear tag to front pen, bottom deck (B1).
Load GROUP 2 - 6 head with **YELLOW RIGHT** and **GREEN LEFT** ear tag to second pen, bottom deck (B2).
Lower ramp
Load GROUP 3 - 6 head with **YELLOW RIGHT** ear tags. 3 **YELLOW LEFT** and 3 **ORANGE LEFT** ear tags to front pen of top deck (T1).
Load GROUP 4 - 6 head with **GREEN RIGHT** & mixed LEFT eartags - 3 **YELLOW LEFT** and 3 **ORANGE LEFT** to rear pen on top deck (T3).
Load GROUP 5 - 6 head with **GREEN RIGHT** ear tags and **GREEN LEFT** ear tags to centre pen of top deck (T2).
*** Check loading order of T2 and T3 with truck driver to suit ramp arrangement.
*** If ramp/pocket and T3 sorts are too hard load the combined 12 as suits and draft back to 2 x 6 on arrival at Saleyard B.
Raise ramp.
Load GROUP 6 - 6 head with **GREEN RIGHT** and **BLUE LEFT** ear tags to rear pen of bottom deck (B3).

TRUCK LOADING ON DEPARTURE **DEPARTURE TIME**



ON ARRIVAL AT SALEYARD B

Unload pen B3.
Lower ramp.
Unload pen T2.
Unload T3/Pocket/Ramp. Saleyard staff will sort by LEFT ear tag colour to BLUE(6), GREEN(6), YELLOW(3) and ORANGE (3).
Each of the 4 groups are not to be mixed with each other. (They will be mixed with cattle from others at Saleyard B).

ON ARRIVAL AT SALEYARD A

Unload pen T1. Saleyard staff will sort by LEFT ear tag to YELLOW(3) and ORANGE (3).
Unload pen B2.
Unload pen B1.
Each of the 4 groups are not to be mixed with each other. (They will be mixed with cattle from others at Saleyard A).

Figure 20. Truck loading instruction for one farm

4.2.4 Phase 2 - Saleyards

The location of the participant farms dictated that the Saleyard B delivery was scheduled prior to Saleyard A. On the evening of June 27th the four trucks, one from each property, drove to Saleyard B and each unloaded 18 head, each as 3 groups of 6. The trucks arrived between 5 and 7 pm which required saleyard access and a stockman to pen the groups as appropriate. Jarrod Lees from MSA supervised this activity and was present to assist and confirm any detail regarding the trial requirements.

The cattle were unloaded into dirt floored covered holding pens and fed hay and water until sale day (June 29th). As unloaded, cattle from all farms were penned as follows on the basis of their left ear tag colour:

12 BLUE LEFT ear tag steers – 6 from Farm 1 and 6 from Farm 2 boxed together.

12 GREEN LEFT ear tag steers – 6 from Farm 1 and 6 from Farm 2 boxed together.

12 Large PINK with small RED LEFT ear tag heifers – 6 from Farm 3 and 6 from Farm 4 boxed together.

12 Large WHITE with small BLUE LEFT ear tag heifers – 6 from Farm 3 and 6 from Farm 4 boxed together.

12 YELLOW LEFT ear tags, 3 from each of the four farms boxed together (6 steers & 6 heifers).

12 ORANGE LEFT ear tags, 3 from each of the four farms boxed together (6 steers & 6 heifers).

The four pens, one from each truck, with mixed YELLOW and ORANGE ear tags were drafted to sort off YELLOW and ORANGE to make up two pens of 12 for each colour.

The four trucks then proceeded to Saleyard B and unloaded the remaining three pens of 6. Dr Peter McGilchrist from Murdoch University was present to assist and confirm detail as required.

Cattle were penned in accordance with their LEFT eartag colour identically to the Saleyard B detail above.

On June 28th prior to the Saleyard A sale the project cattle were exposed to typical saleyard procedures including weighing, paint marking then penned within the sale lanes as shown in Figure 21. As shown in Figure 22 the actual auction moved past the trial pens and the cattle were moved around during sale for buyer observation.

Figure 21 – Typical saleyard conditions

Holly Cuthbertson of Sydney University recorded FLIR footage of each group while in the saleyard pens as shown in Figure 22.

Figure 22 – FLIR readings taken during auction at both saleyards

Post sale the 6 groups, each 12 head of one LEFT ear tag colour (72 head in total), were held separate and fed hay and water until midday on June 29th when they were loaded for transport to the abattoir. Only a single LEFT ear tag colour could be penned together.

On June 29th prior to the Saleyard B sale the 6 eartag colour groups of project cattle were exposed to typical saleyard procedures including weighing, paint marking then penned within the sale lanes. This saleyard had smaller pens resulting in the 12 head groups being further divided into typical sale lots of 4 to 5 head but of common LEFT ear tag colour. The actual auction moved past the trial pens and the cattle were moved around during sale for buyer observation. Holly Cuthbertson of Sydney University recorded FLIR footage of each group while in the saleyard pens.

Post sale the 6 groups, each 12 head of one LEFT ear tag colour (72 head in total), were held separate and loaded as separate groups for transport to the abattoir. Only a single LEFT ear tag colour could be penned together.

On the afternoon of June 29th cattle from both saleyards were transported to the abattoir with trucking arranged for arrival at a similar time. Each left ear tag colour group was maintained in separate pens at the saleyard and during trucking. Vendor declarations were required per normal procedure for a saleyard to abattoir transfer.

Loading was scheduled for midday at Saleyard A and 2:00 pm at Saleyard B. Two double decks were arranged by the abattoir to transport the 72 head from each saleyard. Penning to maintain ear tag colour groups is shown in Figure 23 (The colours in each pen represent the LEFT ear tag colour). The proposed loading provided for the steer penning to be adjusted as loaded according to density with either 3 pens of four or 2 pens of six on the back 3 pens of the bottom deck.

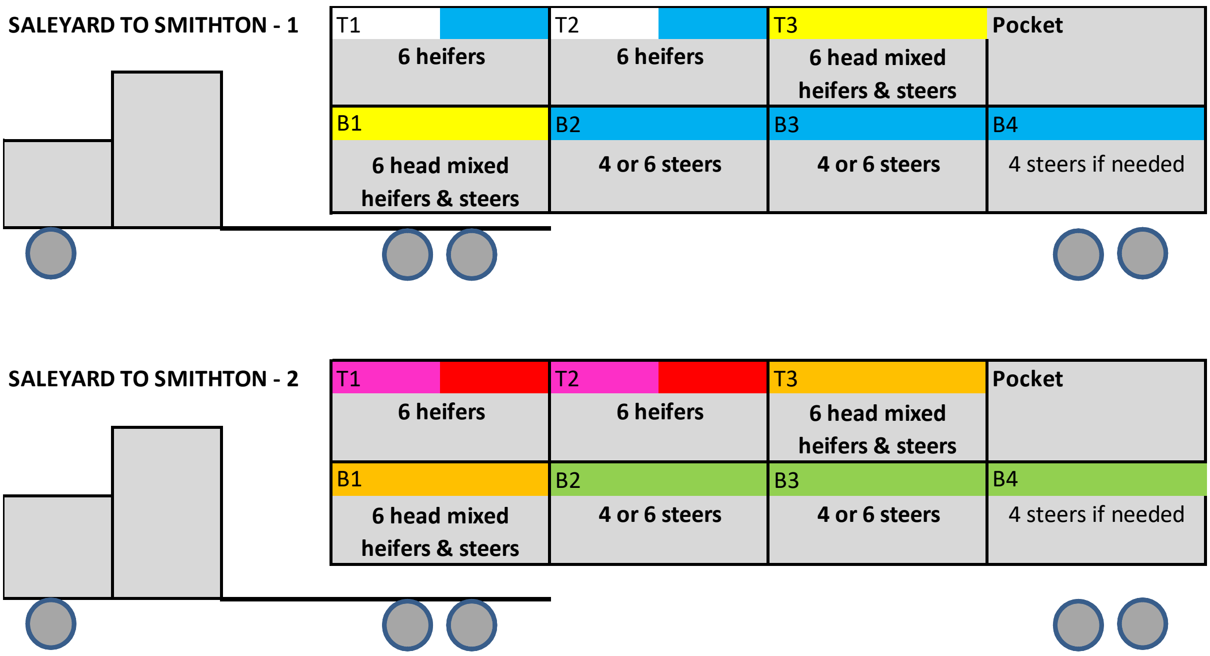


Figure 23. Loading plan for transport from the saleyards to the abattoir

The trucks arrived at the abattoir early in the evening coinciding with the direct consignment cattle from farm deliveries.

4.2.5 Phase 2 - Abattoir final drafting, lairage and holding paddock allocation

On the afternoon of June 29th the research team was present at the abattoir to supervise the unloading of trucks and to ensure that segregation was maintained in initial lairage penning and that cattle to be rested for 14 days were correctly assigned to paddocks in their correct groups.

12 cattle from each of the 4 farms (WHITE RIGHT ear tags) were trucked direct to the abattoir with the trucks arriving early in the evening at a similar time to the saleyard consignments.

Trucks from the two saleyards and 4 farms were unloaded to lairage or to resting paddocks according to the pen plan (as defined by eartag colour) shown in Table 10. Three lairage pens of 24 head each and 4 lairage pens of 12 head each were required for the June 30th kill.

A further 3 holding paddocks for 24 head each were required to rest the balance of the cattle (72 head) for two weeks.

Table 11. Detail of ear tag colours and related abattoir penning instruction for Phase 2 kill 1 cattle

EAR TAG COLOUR								
Right	**Left**	**Penning**	**No**	**Sex**	**Supplier**	**Arriving From**	**Carrier**	**Grouping**
WHITE	RED	Lairage	12	Steer	Farm 1	Farm direct		Individual pen
WHITE	OLIVE GREEN	Lairage	12	Steer	Farm 2	Farm direct		Individual pen
WHITE	Light BLUE	Lairage	12	Heifer	Farm 3	Farm direct		Individual pen
WHITE	PURPLE	Lairage	12	Heifer	Farm 4	Farm direct		Individual pen
GREEN	BLUE	Lairage	12	Steer	Farms 1 & 2	Saleyard A		Box together
YELLOW	BLUE	Lairage	12	Steer	Farms 1 & 2	Saleyard B		
GREEN	GREEN	Paddock	12	Steer	Farms 1 & 2	Saleyard A		Box together
YELLOW	GREEN	Paddock	12	Steer	Farms 1 & 2	Saleyard B		
GREEN	PINK w RED	Lairage	12	Heifer	Farms 3 & 4	Saleyard A		Box together
YELLOW	PINK w RED	Lairage	12	Heifer	Farms 3 & 4	Saleyard B		
GREEN	WHITE w BLUE	Paddock	12	Heifer	Farms 3 & 4	Saleyard A		Box together
YELLOW	WHITE w BLUE	Paddock	12	Heifer	Farms 3 & 4	Saleyard B		
GREEN	YELLOW	Lairage	12	Mixed	All farms	Saleyard A		Box together
YELLOW	YELLOW	Lairage	12	Mixed	All farms	Saleyard B		
GREEN	ORANGE	Paddock	12	Mixed	All farms	Saleyard A		Box together
YELLOW	ORANGE	Paddock	12	Mixed	All farms	Saleyard B		

4.2.6 Phase 2 - Kill 1

The 120 head in lairage were killed on June 30th. The kill order was in 20 groups of 6 (any 6 from the designated ear tag colour pen) as detailed in Table 12 to ensure treatments were spread across the kill.

Kill procedures were identical to those in Phase 1 with FLIR recording by two cameras placed over and beside the lead up race and a third above the knocking box. Two retinal cameras were placed within the knocking box. Blood samples were collected in the bleed area and eartag to carcase number correlation was recorded. MSA staff recorded hourly pH and temperature for 5 hours post chiller entry to establish declines.

Table 12. Treatment and source group allocation to Phase 2 Kill 1

Kill Order	LEFT eartag	Treatment	No Head	MSA
1	RED	Never Mixed Steers	6	Yes
2	YELLOW	Mixed Sex	6	
3	Light BLUE	Never Mixed Heifers	6	Yes
4	PINK w RED	Mixed Heifers	6	
5	BLUE	Mixed Steers	6	
6	PURPLE	Never Mixed Heifers	6	Yes
7	OLIVE GREEN	Never Mixed Steers	6	Yes
8	BLUE	Mixed Steers	6	
9	YELLOW	Mixed Sex	6	
10	PINK w RED	Mixed Heifers	6	
11	BLUE	Mixed Steers	6	
12	Light BLUE	Never Mixed Heifers	6	Yes
13	PINK w RED	Mixed Heifers	6	
14	RED	Never Mixed Steers	6	Yes
15	YELLOW	Mixed Sex	6	
16	PINK w RED	Mixed Heifers	6	
17	BLUE	Mixed Steers	6	
18	YELLOW	Mixed Sex	6	
19	PURPLE	Never Mixed Heifers	6	Yes
20	OLIVE GREEN	Never Mixed Steers	6	Yes

On July 1st all trial carcases were MSA graded and selected cuts were collected for consumer testing following identical procedures to those described for Phase 1.

4.2.7 Phase 2 - Kill 2

On July 13th the final 12 head from each of the 4 farms were trucked to the abattoir in the late afternoon and held in single lairage pens (4 pens of 12). The saleyard cattle that had been rested for 2 weeks were also moved to lairage at a similar time requiring lairage space for 3 groups of 24 as depicted in Table 13.

Table 13. Lairage penning for Phase 2 Kill 2

EAR TAG COLOUR							
Right	**Left**	**Penning**	**No**	**Sex**	**Supplier**	**Arriving From**	**Carrier**
ORANGE	RED	Lairage	12	Steer	Farm 1	Farm direct	
ORANGE	OLIVE GREEN	Lairage	12	Steer	Farm 2	Farm direct	
ORANGE	Light BLUE	Lairage	12	Heifer	Farm 3	Farm direct	
ORANGE	PURPLE	Lairage	12	Heifer	Farm 4	Farm direct	
GREEN	GREEN	Lairage	12	Steer	Farms 1 & 2	Saleyard A	Group of 24 ex paddock
YELLOW	GREEN	Lairage	12	Steer	Farms 1 & 2	Saleyard B	
GREEN	WHITE w BLUE	Lairage	12	Heifer	Farms 3 & 4	Saleyard A	Group of 24 ex paddock
YELLOW	WHITE w BLUE	Lairage	12	Heifer	Farms 3 & 4	Saleyard B	
GREEN	ORANGE	Lairage	12	Mixed	All farms	Saleyard A	Group of 24 ex paddock
YELLOW	ORANGE	Lairage	12	Mixed	All farms	Saleyard B	

On July 14th the cattle received direct from the 4 farms (48) and those rested for two weeks ex the saleyards (72) were killed following identical procedures to those in previous kills. The kill order drew 20 groups of 6 head (any six) from pens in the order shown in Table 14 to ensure each treatment group (defined by LEFT ear tag colour) was distributed across the kill.

Table 14. Designated kill order for Phase 2 kill 2

LEFT eartag	Treatment	No Head
RED	Never Mixed Steers	6
YELLOW	Mixed Sex	6
Light BLUE	Never Mixed Heifers	6
PINK w RED	Mixed Heifers	6
BLUE	Mixed Steers	6
PURPLE	Never Mixed Heifers	6
OLIVE GREEN	Never Mixed Steers	6
BLUE	Mixed Steers	6
YELLOW	Mixed Sex	6
PINK w RED	Mixed Heifers	6
BLUE	Mixed Steers	6
Light BLUE	Never Mixed Heifers	6
PINK w RED	Mixed Heifers	6
RED	Never Mixed Steers	6
YELLOW	Mixed Sex	6
PINK w RED	Mixed Heifers	6
BLUE	Mixed Steers	6
YELLOW	Mixed Sex	6
PURPLE	Never Mixed Heifers	6
OLIVE GREEN	Never Mixed Steers	6

Kill procedures were identical to those in Phase 1 with FLIR recording by two cameras placed over and beside the lead up race and a third above the knocking box. Two retinal cameras were placed within the knocking box. Blood samples were collected in the bleed area and eartag to carcase number correlation was recorded. MSA staff recorded hot ossification and hump height as well as hourly pH and temperature for 5 hours post chiller entry to establish declines.

On July 15th 2016 all trial carcases were MSA graded in preparation for cut collection at boning. As with the three previous kills HunterLab and NIX colour measurements were recorded for each carcase and images were taken with the Kuchida MIJ camera.

Additional cuts were obtained in this collection to facilitate a dry and wet ageing comparison to be conducted by Murdoch and Melbourne University researchers with consumer testing planned in both Australia and Japan. The dry aged study required the collection of bone in OP ribs and loins from 24 carcases. The 24 head, 16 steers and 8 heifers, were selected from the “never mixed” trial groups with further screening to include heavier carcases and normal pH.

Whereas the standard procedure for all the King Island and saleyard project carcases was to collect the striploin, tenderloin, outside flat, eye round and oyster blade primals from a single carcase side a matching bone in rib and loin was required for the 24 carcases to be utilised in the dry ageing study. The bone in ribs were included to provide a larger portion of *M.longissimus* (LD) muscle for additional dry and wet aged comparison and also reflected their common use in dry ageing. Alternative left and right sides were elected for bone in and boneless cuts with a balanced 12 of each.

To add anticipated eating quality range to the Japanese/Australian consumer comparison it was elected to also collect oyster blade and outside primals from both sides of the selected 24 bodies. A further complication was the requirement for product destined for Japan to remain in an export registered establishment at all times. As the UNE facility was not registered arrangements were made to transfer all cuts from the 24 bodies to a Japan licensed premise located on the Queensland Gold Coast that also had a dry ageing facility. Further detail on the dry ageing activity is provided in section 5.3.

Amended cut ticketing was developed for the selected 24 bodies with colour coding again utilised to draw attention to the different cutting lines and packing required. The wet aged cuts were vacuum packed as normal whereas the bone in cuts to be dry aged were boxed in plastic liners for transport. The standard 5 cuts were collected from the left sides of the 96 bodies not included in the dry aged research and vacuum packed with their primal ID following identical protocol to the previous 3 project cut collections.

All cuts produced were transported to the abattoir Melbourne distribution centre following previous protocol other than including an export meat transfer certificate for the export product. The shipment was collected by Polkinghornes and transported to the Gold Coast and UNE respectively by refrigerated transport.

4.2.8 Blood and IRT data collection and analysis

As detailed above field IRT measures were taken at multiple points from farm to slaughter in both project phases. Blood samples were collected from all cattle at slaughter.

Detailed description of the collection and analysis methodologies is provided by Loudon et.al (2019a and 2019b) for the blood work and by Cuthbertson et.al (2019a, 2019b, 2019c and 2019d)

5 Methodology – Cut up and consumer sample fabrication

Phase 1 and 2 of the project utilised a common base design of comparing grilled samples from 5 muscles after 7 and 21 days post mortem ageing for all carcases.

The cuts collected and muscles utilised in consumer sample fabrication are displayed in Table 15.

Table 15. Cuts collected and muscles fabricated to MSA consumer samples

Primal	HAM no.	MSA Code	Muscle
Tenderloin	**2150**	TDR062	M.psoas major
Striploin	2142	STR045	M.longissimus dorsi et lumorum
Cuberoll **	2243	CUB045	M.longissimus dorsi et thoracis
		CUB081	M.spinalis dorsi
Eye Round	2040	EYE075	M.semitendinosus
Outside Flat	2050	OUT005	M.biceps femoris
Oyster Blade	2304	OYS036	M.infraspinatus
** Cube roll only collected from selected Phase 2, kill 2, farm direct groups and utilised in paired Japanese dry ageing study			

To add further value a number of additional treatments were included where there was sufficient product beyond the base design requirement. These were:

1. Phase 1, kill 1 (Directly off the ships)

   – Ageing to 21 days in a "block" versus standard MSA sliced steak form and extended block ageing to 42 days as reported in L.EQT.1629

2. Phase 1, kill 2 (After 2 weeks post shipping rest)

   – Stable versus erratic temperature to 21 days ageing to approximate Domestic versus Export ageing conditions.

   – Extended ageing of all cuts to 44, 63 and 84 days post slaughter.

3. Phase 2, kill 1 (Direct from farms and saleyards)

   – Extended ageing for eye round, outside flat and striploin to 49, 56,70 and 77 days post slaughter.

4. Phase 2, kill 2 (After 2 weeks rest post arrival from farm or saleyard)

   – Extended ageing for eye round, outside flat and striploin to 42, 49, 56, 63, 70, 77 and 84 days post slaughter.

   – Dry bone-in and wet boneless ageing of striploin and cube roll cuts with consumer testing by Japanese consumers in conjunction with Japanese beef cuts. Dry versus wet aged comparisons were at 35 and 56 days ageing with a further 21 days wet followed by 35 days dry aged (total 56 days) comparison as reported by Warner et al. (2017).

5.1 MSA Cut-up design and primal cut collection management

Standard MSA protocol (Anon, 2008) was utilised throughout the trial design process and implemented in cut fabrication into consumer samples for the core project and related experiments. In brief, the design process was initiated through MSA Cut Up Developer (CUD) software. The CUD software provided a structure to maintain unique and interlinked identification at all points from the live animal to final consumer plate labels together with work instruction and recording sheets. An example of the initial CUD nomination of groups, numbers of sides and cuts to be collected is displayed in Figure dd.

Jump to Model | Jump to Display Cut Up | Jump to Input sht | Create and Install Body Sides | Expand Kill into Model for Cuts

Master Group Number	KILL (Group)	Kill Date	Abattoir or Works	Groups Incorp.	Groups Solo	Error Flags
481	482	Mon 06 Jun 16	Greenham Tasmania		80	
				Total Sides	Total Sides	

GROUP DESC	GROUP	No. of SIDES		L or R or LR or L/R	HANG	STIM	TDR034	TDR062	TDR063	CUB081	CUB045	STR045	OYS036	BLD095	BLD096	CTR085	RMP131	RMP231	RMP005	RMP032	RMP087	KNU066	KNU098	KNU099	KNU100	OUT005	OUT029	EYE075	TOP001	TOP033	TOP073
Never Mixed Steers	482.1	16		L	AT	LVES		y				y	y													y		y			
Never Mixed Heifers	482.2	16		L	AT	LVES		y				y	y													y		y			
Mixed Steers	482.3	16		L	AT	LVES		y				y	y													y		y			
Mixed Heifers	482.4	16		L	AT	LVES		y				y	y													y		y			
Mixed Sex	482.5	16		L	AT	LVES		y				y	y													y		y			
Group Totals of BodySides		80																													

Remember - All Group tally numbers are to be counted in sides - NOT bodies. In groups with L & R sides, allow for both sides in BodySide counts.

Your Name for This Cut Up File	King Island 2nd Kill

Figure 24. CUD design table detailing broad group, side numbers and cuts to be collected

The base design shown in Figure 24 was then expanded to detail cut positions, cook and ageing parameters as displayed in Figure 25. As shown in Figure dd1 the 5 cuts were allocated either 3 or 4 positions according to their expected size. All cuts were prepared as grills for consumer evaluation but to trigger a separate label for “Link” product (served as a first sample to consumers) and for samples designated to be aged with temperature variation, loosely analogous to a domestic butcher shop environment, a LNK and DOM cooking method was utilised in the CUD process. A base 4 ageing periods were also specified for each muscle.

From these entries a further design sheet was generated by the CUD software as illustrated in Figure 26. The example shows a portion of the display rearranged to illustrate the allocation of ageing to cut position across some common bodies. Over the entire design the within cut positions and ageing treatments were rotated to ensure cut position was not confounded with the ageing treatments. The pseudo “cooks” of LNK and DOM are also shown with position allocations. The actual cook was modified to grill (GRL) at a later process stage. The link product was always allocated to the posterior (P4) striploin position as this position is flatter and more different to the A1, A2 and P3 positions which are more consistent and reserved for the core 7 and 21 day ageing in this project.

Jump to Kill | Jump to Display Cut Up | Test Model as Cut Up | Publish Acquisition Sheet

MSTR GROUP	GROUP	GROUP COMMENT	DEG FRD	CELL QTY	SIDES	CUT NAME	SUM POS	POS 1	POS 2	POS 3	POS 4	SUM COOKS	ROTATE COOKS	GRL	LNK	DOM	FLV	SC2	CRN	No. AGES	ROTATE AGES	AGE 1	AGE 2	AGE 3	AGE 4
481	482.1	Never Mixed Steers	48	16	16	TDR062	3	H	C	T		4		3		1				4		7	21	42	63
481	482.1	Never Mixed Steers	64	16	16	STR045	4	A1	A2	P3	P4	4		2	1	1				4		7	21	42	63
481	482.1	Never Mixed Steers	48	16	16	OYS036	3	H	C	T		3		2		1				4		7	21	42	63
481	482.1	Never Mixed Steers	64	16	16	OUT005	4	H1	H2	T3	T4	4		3		1				4		7	21	42	63
481	482.1	Never Mixed Steers	48	16	16	EYE075	3	H	C	T		4		2	1	1				4		7	21	42	63
481	482.2	Never Mixed Heifers	48	16	16	TDR062	3	H	C	T		4		3		1				4		7	21	42	63
481	482.2	Never Mixed Heifers	64	16	16	STR045	4	A1	A2	P3	P4	4		2	1	1				4		7	21	42	63
481	482.2	Never Mixed Heifers	48	16	16	OYS036	3	H	C	T		3		2		1				4		7	21	42	63
481	482.2	Never Mixed Heifers	64	16	16	OUT005	4	H1	H2	T3	T4	4		3		1				4		7	21	42	63
481	482.2	Never Mixed Heifers	48	16	16	EYE075	3	H	C	T		4		2	1	1				4		7	21	42	63
481	482.3	Mixed Steers	48	16	16	TDR062	3	H	C	T		4		3		1				4		7	21	42	63
481	482.3	Mixed Steers	64	16	16	STR045	4	A1	A2	P3	P4	4		2	1	1				4		7	21	42	63
481	482.3	Mixed Steers	48	16	16	OYS036	3	H	C	T		3		2		1				4		7	21	42	63
481	482.3	Mixed Steers	64	16	16	OUT005	4	H1	H2	T3	T4	4		3		1				4		7	21	42	63
481	482.3	Mixed Steers	48	16	16	EYE075	3	H	C	T		4		2	1	1				4		7	21	42	63
481	482.4	Mixed Heifers	48	16	16	TDR062	3	H	C	T		4		3		1				4		7	21	42	63
481	482.4	Mixed Heifers	64	16	16	STR045	4	A1	A2	P3	P4	4		2	1	1				4		7	21	42	63
481	482.4	Mixed Heifers	48	16	16	OYS036	3	H	C	T		3		2		1				4		7	21	42	63
481	482.4	Mixed Heifers	64	16	16	OUT005	4	H1	H2	T3	T4	4		3		1				4		7	21	42	63
481	482.4	Mixed Heifers	48	16	16	EYE075	3	H	C	T		4		2	1	1				4		7	21	42	63
481	482.5	Mixed Heifers	48	16	16	TDR062	3	H	C	T		4		3		1				4		7	21	42	63
481	482.5	Mixed Heifers	64	16	16	STR045	4	A1	A2	P3	P4	4		2	1	1				4		7	21	42	63
481	482.5	Mixed Heifers	48	16	16	OYS036	3	H	C	T		3		2		1				4		7	21	42	63
481	482.5	Mixed Heifers	64	16	16	OUT005	4	H1	H2	T3	T4	4		3		1				4		7	21	42	63
481	482.5	Mixed Heifers	48	16	16	EYE075	3	H	C	T		4		2	1	1				4		7	21	42	63

Figure 25. CUD input to extend design to cut position, cook methods and ageing specification

Grp	Body	Cut	Cook	Age	Pos	Side	Cmpr	OBJ
482.1	1	TDR062	GRL	7	H	L	Ck	y
482.1	1	TDR062	GRL	21	C	L	Ck	y
482.1	1	TDR062	DOM	21	T	L	Ck	y
482.1	2	TDR062	GRL	21	H	L	Ck	y
482.1	2	TDR062	DOM	21	C	L	Ck	y
482.1	2	TDR062	GRL	7	T	L	Ck	y
482.1	3	TDR062	DOM	21	H	L	Ck	y
482.1	3	TDR062	GRL	7	C	L	Ck	y
482.1	3	TDR062	GRL	21	T	L	Ck	y
482.1	4	TDR062	GRL	21	H	L	Ck	y
482.1	4	TDR062	GRL	7	C	L	Ck	y
482.1	4	TDR062	DOM	21	T	L	Ck	y
482.1	5	TDR062	GRL	63	H	L	Ck	y
482.1	5	TDR062	GRL	7	C	L	Ck	y
482.1	5	TDR062	GRL	21	T	L	Ck	y
482.1	6	TDR062	GRL	7	H	L	Ck	y
482.1	6	TDR062	GRL	42	C	L	Ck	y
482.1	6	TDR062	GRL	21	T	L	Ck	y
482.1	7	TDR062	GRL	21	H	L	Ck	y
482.1	7	TDR062	GRL	7	C	L	Ck	y
482.1	7	TDR062	GRL	84	T	L	Ck	y
482.1	8	TDR062	GRL	84	H	L	Ck	y
482.1	8	TDR062	GRL	21	C	L	Ck	y
482.1	8	TDR062	GRL	7	T	L	Ck	y
482.1	9	TDR062	GRL	7	H	L	Ck	y
482.1	9	TDR062	GRL	21	C	L	Ck	y
482.1	9	TDR062	GRL	42	T	L	Ck	y
482.1	10	TDR062	GRL	21	H	L	Ck	y
482.1	10	TDR062	GRL	63	C	L	Ck	y
482.1	10	TDR062	GRL	7	T	L	Ck	y
482.1	11	TDR062	GRL	42	H	L	Ck	y
482.1	11	TDR062	GRL	7	C	L	Ck	y

Grp	Body	Cut	Cook	Age	Pos	Side	Cmpr	OBJ
482.1	1	STR045	GRL	21	A1	L	Ck	y
482.1	1	STR045	DOM	21	A2	L	Ck	y
482.1	1	STR045	GRL	7	P3	L	Ck	y
482.1	1	STR045	LNK	7	P4	L	Ck	
482.1	2	STR045	DOM	21	A1	L	Ck	y
482.1	2	STR045	GRL	7	A2	L	Ck	y
482.1	2	STR045	GRL	21	P3	L	Ck	y
482.1	2	STR045	LNK	7	P4	L	Ck	
482.1	3	STR045	GRL	7	A1	L	Ck	y
482.1	3	STR045	DOM	21	A2	L	Ck	y
482.1	3	STR045	GRL	21	P3	L	Ck	y
482.1	3	STR045	LNK	7	P4	L	Ck	
482.1	4	STR045	GRL	21	A1	L	Ck	y
482.1	4	STR045	GRL	7	A2	L	Ck	y
482.1	4	STR045	DOM	21	P3	L	Ck	y
482.1	4	STR045	LNK	7	P4	L	Ck	
482.1	5	STR045	GRL	7	A1	L	Ck	
482.1	5	STR045	GRL	42	A2	L	Ck	
482.1	5	STR045	GRL	21	P3	L	Ck	
482.1	5	STR045	LNK	7	P4	L	Ck	
482.1	6	STR045	GRL	21	A1	L	Ck	
482.1	6	STR045	GRL	7	A2	L	Ck	
482.1	6	STR045	GRL	63	P3	L	Ck	
482.1	6	STR045	LNK	7	P4	L	Ck	
482.1	7	STR045	GRL	84	A1	L	Ck	
482.1	7	STR045	GRL	21	A2	L	Ck	
482.1	7	STR045	GRL	7	P3	L	Ck	
482.1	7	STR045	LNK	7	P4	L	Ck	
482.1	8	STR045	GRL	7	A1	L	Ck	
482.1	8	STR045	GRL	21	A2	L	Ck	
482.1	8	STR045	GRL	42	P3	L	Ck	
482.1	8	STR045	LNK	7	P4	L	Ck	

Grp	Body	Cut	Cook	Age	Pos	Side	Cmpr	OBJ
482.1	1	OYS036	GRL	7	H	L	Ck	y
482.1	1	OYS036	GRL	21	C	L	Ck	y
482.1	1	OYS036	DOM	21	T	L	Ck	y
482.1	2	OYS036	GRL	21	H	L	Ck	y
482.1	2	OYS036	DOM	21	C	L	Ck	y
482.1	2	OYS036	GRL	7	T	L	Ck	y
482.1	3	OYS036	DOM	21	H	L	Ck	y
482.1	3	OYS036	GRL	7	C	L	Ck	y
482.1	3	OYS036	GRL	21	T	L	Ck	y
482.1	4	OYS036	DOM	21	H	L	Ck	y
482.1	4	OYS036	GRL	21	C	L	Ck	y
482.1	4	OYS036	GRL	7	T	L	Ck	y
482.1	5	OYS036	GRL	42	H	L	Ck	y
482.1	5	OYS036	GRL	7	C	L	Ck	y
482.1	5	OYS036	GRL	21	T	L	Ck	y
482.1	6	OYS036	GRL	7	H	L	Ck	y
482.1	6	OYS036	GRL	63	C	L	Ck	y
482.1	6	OYS036	GRL	21	T	L	Ck	y
482.1	7	OYS036	GRL	21	H	L	Ck	y
482.1	7	OYS036	GRL	7	C	L	Ck	y
482.1	7	OYS036	GRL	84	T	L	Ck	y
482.1	8	OYS036	GRL	63	H	L	Ck	y
482.1	8	OYS036	GRL	21	C	L	Ck	y
482.1	8	OYS036	GRL	7	T	L	Ck	y
482.1	9	OYS036	GRL	7	H	L	Ck	y
482.1	9	OYS036	GRL	21	C	L	Ck	y
482.1	9	OYS036	GRL	42	T	L	Ck	y
482.1	10	OYS036	GRL	21	H	L	Ck	y
482.1	10	OYS036	GRL	84	C	L	Ck	y
482.1	10	OYS036	GRL	7	T	L	Ck	y
482.1	11	OYS036	GRL	84	H	L	Ck	y
482.1	11	OYS036	GRL	7	C	L	Ck	y

Grp	Body	Cut	Cook	Age	Pos	Side	Cmpr	OBJ
482.1	1	OUT005	GRL	7	H1	L	Ck	y
482.1	1	OUT005	DOM	21	H2	L	Ck	y
482.1	1	OUT005	GRL	21	T3	L	Ck	y
482.1	1	OUT005	GRL	21	T4	L	Ck	
482.1	2	OUT005	GRL	21	H1	L	Ck	y
482.1	2	OUT005	GRL	7	H2	L	Ck	y
482.1	2	OUT005	DOM	21	T3	L	Ck	y
482.1	2	OUT005	DOM	21	T4	L	Ck	
482.1	3	OUT005	DOM	21	H1	L	Ck	y
482.1	3	OUT005	GRL	21	H2	L	Ck	y
482.1	3	OUT005	GRL	7	T3	L	Ck	y
482.1	3	OUT005	GRL	7	T4	L	Ck	
482.1	4	OUT005	DOM	21	H1	L	Ck	y
482.1	4	OUT005	GRL	7	H2	L	Ck	y
482.1	4	OUT005	GRL	21	T3	L	Ck	y
482.1	4	OUT005	GRL	21	T4	L	Ck	
482.1	5	OUT005	GRL	21	H1	L	Ck	y
482.1	5	OUT005	GRL	7	H2	L	Ck	y
482.1	5	OUT005	GRL	42	T3	L	Ck	y
482.1	5	OUT005	GRL	42	T4	L	Ck	
482.1	6	OUT005	GRL	63	H1	L	Ck	y
482.1	6	OUT005	GRL	21	H2	L	Ck	y
482.1	6	OUT005	GRL	7	T3	L	Ck	y
482.1	6	OUT005	GRL	7	T4	L	Ck	
482.1	7	OUT005	GRL	84	H1	L	Ck	y
482.1	7	OUT005	GRL	7	H2	L	Ck	y
482.1	7	OUT005	GRL	21	T3	L	Ck	y
482.1	7	OUT005	GRL	21	T4	L	Ck	
482.1	8	OUT005	GRL	21	H1	L	Ck	y
482.1	8	OUT005	GRL	42	H2	L	Ck	y
482.1	8	OUT005	GRL	7	T3	L	Ck	y
482.1	8	OUT005	GRL	7	T4	L	Ck	

Grp	Body	Cut	Cook	Age	Pos	Side	Cmpr	OBJ
482	1	EYE075	GRL	7	H	L	Ck	y
482	1	EYE075	GRL	21	C	L	Ck	y
482	1	EYE075	DOM	21	T	L	Ck	y
482	2	EYE075	GRL	21	H	L	Ck	y
482	2	EYE075	DOM	21	C	L	Ck	y
482	2	EYE075	GRL	7	T	L	Ck	y
482	3	EYE075	DOM	21	H	L	Ck	y
482	3	EYE075	GRL	7	C	L	Ck	y
482	3	EYE075	GRL	21	T	L	Ck	y
482	4	EYE075	DOM	21	H	L	Ck	y
482	4	EYE075	GRL	21	C	L	Ck	y
482	4	EYE075	GRL	7	T	L	Ck	y
482	5	EYE075	GRL	84	H	L	Ck	y
482	5	EYE075	GRL	7	C	L	Ck	y
482	5	EYE075	GRL	21	T	L	Ck	y
482	6	EYE075	GRL	7	H	L	Ck	y
482	6	EYE075	GRL	42	C	L	Ck	y
482	6	EYE075	GRL	21	T	L	Ck	y
482	7	EYE075	GRL	21	H	L	Ck	y
482	7	EYE075	GRL	7	C	L	Ck	y
482	7	EYE075	GRL	63	T	L	Ck	y
482	8	EYE075	GRL	42	H	L	Ck	y
482	8	EYE075	GRL	21	C	L	Ck	y
482	8	EYE075	GRL	7	T	L	Ck	y
482	9	EYE075	GRL	7	H	L	Ck	y
482	9	EYE075	GRL	21	C	L	Ck	y
482	9	EYE075	GRL	84	T	L	Ck	y
482	10	EYE075	GRL	21	H	L	Ck	y
482	10	EYE075	GRL	63	C	L	Ck	y
482	10	EYE075	GRL	7	T	L	Ck	y
482	11	EYE075	GRL	63	H	L	Ck	y
482	11	EYE075	GRL	7	C	L	Ck	y

Figure 26. CUD display for allocated balance of position and ageing within 5 cuts (layout modified for display purposes)

The “Y” in the OBJ columns signifies that an objective label was required to identify samples desired for laboratory analysis. When a final balanced design was completed by manually adjusting the cook, and ageing treatments within cut and position the CUD software was utilised to generate an

"Acquisition Sheet", used to record the eartag, carcase number and Primal ID assigned to each cut during cut collection as described in section 3.2.9. The unique allocated Primal numbers were utilised to produce the Primal labels illustrated in Figure 18. The completed sheet, a portion of which is displayed in Figure 27, was then utilised in a further CUD procedure to generate control sheets and sample labels, detailed in section 5.2.

GroupComment	Group	AnimalID	CUD ref	Works Body No.	Side	Hang	Stim	Primal	Primal ID
Never Mixed Steers	482.1	58	1	256	L	AT	LVES	TDR	50403
Never Mixed Steers	482.1		1		L	AT	LVES	STR	50404
Never Mixed Steers	482.1		1		L	AT	LVES	OYS	50405
Never Mixed Steers	482.1		1		L	AT	LVES	OUT	50406
Never Mixed Steers	482.1		1		L	AT	LVES	EYE	50407
Never Mixed Steers	482.1	35	2	257	L	AT	LVES	TDR	50408
Never Mixed Steers	482.1		2		L	AT	LVES	STR	50409
Never Mixed Steers	482.1		2		L	AT	LVES	OYS	50410
Never Mixed Steers	482.1		2		L	AT	LVES	OUT	50411
Never Mixed Steers	482.1		2		L	AT	LVES	EYE	50412
Never Mixed Steers	482.1	13	3	258	L	AT	LVES	TDR	50413
Never Mixed Steers	482.1		3		L	AT	LVES	STR	50414
Never Mixed Steers	482.1		3		L	AT	LVES	OYS	50415
Never Mixed Steers	482.1		3		L	AT	LVES	OUT	50416
Never Mixed Steers	482.1		3		L	AT	LVES	EYE	50417

Figure 27. Portion of Acquisition Primal sheet with animal, body and Primal ID

5.2 MSA Consumer Sample Fabrication

As noted the collected primal cuts from each of the phase 1 and phase 2 kills were shipped from Tasmania to Melbourne then transported by road to UNE for further fabrication to consumer samples. There was a modification for a portion of the final (Phase 2, kill 2) collection due to some product needing to retain export status to enable shipment to Japan. For this kill product was separated during shipping from Tasmania and within the truck into export and domestic status. The export product was delivered to an export registered facility on the Gold Coast as described in section 5.3 with the domestic product delivered to UNE.

The following procedures were followed during fabrication at both sites and for each of the 4 collections.

After confirming the final Acquisition sheet details a CUD procedure was utilised to produce a CutUpFile which contained a control sheet, partially reproduced in Figure 28, and Avery 21 up labels for identification of the fabricated samples, with examples shown in Figure 29.

The CutUpSheet control format was in Primal ID order with each of the samples to be fabricated aligned with the primal ID, together with the type of sample to be fabricated (Cook), the muscle position to be utilised (Pos) and the designated days of ageing (Age). The Seq (Sequence) and EQS (EQSRef) were unique sample identifiers that were retained through to the final consumer record. These were allocated by CUD software from a label resource that ensured that none could be duplicated.

Seq	EQS	Primal	Cut	Cook	Age	Pos	Kill	Obj	Check
AUS87642	B7V9	50403	TDR062	DOM	21	T	Mon 06 Jun 16	y	
AUS87747	J3N1	50403	TDR062	GRL	7	H	Mon 06 Jun 16	y	
AUS87748	M1V6	50403	TDR062	GRL	21	C	Mon 06 Jun 16	y	
AUS87643	N5W9	50404	STR045	DOM	21	A2	Mon 06 Jun 16	y	
AUS87749	P8Y1	50404	STR045	GRL	21	A1	Mon 06 Jun 16	y	
AUS87750	T5J7	50404	STR045	GRL	7	P3	Mon 06 Jun 16	y	
AUS88922	Q5G8	50404	STR045	LNK	7	P4	Mon 06 Jun 16		
AUS87644	J0R1	50405	OYS036	DOM	21	T	Mon 06 Jun 16	y	
AUS87751	U7D8	50405	OYS036	GRL	7	H	Mon 06 Jun 16	y	
AUS87752	N8P4	50405	OYS036	GRL	21	C	Mon 06 Jun 16	y	
AUS87645	E8V7	50406	OUT005	DOM	21	H2	Mon 06 Jun 16	y	
AUS87753	M6B1	50406	OUT005	GRL	7	H1	Mon 06 Jun 16	y	
AUS87754	H9N6	50406	OUT005	GRL	21	T3	Mon 06 Jun 16	y	
AUS87755	U5M7	50406	OUT005	GRL	21	T4	Mon 06 Jun 16		
AUS87646	N1E6	50407	EYE075	DOM	21	T	Mon 06 Jun 16	y	
AUS87756	N0U8	50407	EYE075	GRL	7	H	Mon 06 Jun 16	y	
AUS87757	W1T0	50407	EYE075	GRL	21	C	Mon 06 Jun 16	y	
AUS87647	N2V0	50408	TDR062	DOM	21	C	Mon 06 Jun 16	y	
AUS87758	T6K6	50408	TDR062	GRL	21	H	Mon 06 Jun 16	y	
AUS87759	G8N5	50408	TDR062	GRL	7	T	Mon 06 Jun 16	y	

Figure 28. Portion of CUD CutUpSheet control sheet displaying individual samples for one body.

50403	OBJECTIVE 50403 - TDR062 B7V9 2706	AUS87642 B7V9 50403 DOM T TDR062 2706
OBJECTIVE 50403 - TDR062 J3N1 1306	AUS87747 J3N1 50403 GRL H TDR062 1306	OBJECTIVE 50403 - TDR062 M1V6 2706
AUS87748 M1V6 50403 GRL C TDR062 2706	50404	OBJECTIVE 50404 - STR045 N5W9 2706
AUS87643 N5W9 50404 DOM A2 STR045 2706	OBJECTIVE 50404 - STR045 P8Y1 2706	AUS87749 P8Y1 50404 GRL A1 STR045 2706
OBJECTIVE 50404 - STR045 T5J7 1306	AUS87750 T5J7 50404 GRL P3 STR045 1306	AUS88922 Q5G8 50404 LNK P4 STR045 1306

Figure 29. Portion of sample labels produced by CUD software

Figure 29 displays the CUD label format that was printed onto Avery 7165 21 up stock. The portion shown displays labels for the first two primal numbers shown in the control sheet (Figure 28). The Primal ID, 50403 and 50404 in the example, is followed by labels for each sample to be produced with the Sequence and EQSRef codes, the source Primal No and MSA code, TDR062 and STR045 in the example, the position each is be prepared from, T = Tail, H = Head and C = Centre for the TDR062, and a freeze down date, 2706 and 1306 in the example, to achieve the designated ageing.

The physical cut-up procedure was as follows:

1. The transported cuts were held in their cartons at 1 to 3˚C prior to fabrication.
2. As required a carton was opened, a single cut removed and its vacuum packaging removed. The cut was then placed on a plastic tray with the Primal ID label, packed within the vacuum bag during collection at the abattoir, placed in the top left tray corner to retain ID.
3. The cut was then fully denuded by a butcher and divided into component muscles where applicable (Cube roll and Tenderloin). All surface fat and epimysium was removed together with portions of other muscles to leave a single muscle portion or portions. (This included

removal of the tenderloin head (*M.illiacus*) and separation of the *Mm.longissimus dorsi* and *spinalis dorsi* for the cube rolls processed at the Gold Coast facility).

Figure 30. Muscles were denuded and divided by butchers, maintaining ID with labels

4. The denuded muscle(s) were then placed back on the tray which was passed to the recording position.
5. The Primal ID was then referenced in the CutUpSheet and Label files. The cut was oriented in a standard form on the tray and the Avery labels lightly attached to the tray edge adjacent to the nominated position (H, C, T for TDR062, OYS036 and EYE075, A1, A2, P3, P4 and H1, H2, T3 and T4 for striploin and outside flat respectively). The labels provided an instruction for subsequent fabrication.
6. The trays were then passed to a cutting station equipped with an adjustable cutting jig, pre-set to 25mm.
7. A butcher then placed the cut on the jig in the designated orientation with the grain direction at right angles to the cutting board stop. A facing piece was removed to leave an even face and then progressive 25mm slices cut using the jig to ensure a consistent thickness and parallel faces.

Figure 31. Muscle on cutting jig prior to removing the facing piece

8. The slices were laid out in order of cutting to maintain position and trimmed to MSA specified grill samples of approximately 38 x 65mm. Five sample steaks were prepared for each label from the nominated cut position. Suitable left over muscle portions were allocated to Objective labels.
9. Each of the 5 steaks per sample were wrapped in freezer wrap (to prevent them sticking together during freezing) and placed within a 200 x 250mm vacuum pouch with the label affixed.
10. The sample was then vacuum sealed and sorted to Styrofoam boxes in accordance with the Freeze date.
11. Cartons of packed product were held chilled at 0 to 4˚C until reaching the freeze data at which point they were removed from the Styrofoam boxes and laid out single depth for freezing. After freezing they were repacked in Styrofoam for storage at -20˚C.
12. Any samples that could not be fabricated due to muscle size were noted in the CutUpSheet and all those successfully fabricated marked.
13. After the CutUp was completed the CutUpSheet file was checked against the label sheets to cross check for any missing samples (labels still retained in the bound label file) and the file transferred to the AUSBlue database utilising a final CUD software routine that also transferred all available information in the CUD file (Group, carcase, primal, sequence and EQSRef ID plus abattoir and kill date, hang, stimulation, cook, days aged and muscle position).
14. The transfer routine ensured only actual samples were transferred to AUSBlue where they were assigned an "Available" status enabling them to be picked for sensory testing.

Table 16 presents a breakdown of the source groups, treatments, muscles and days ageing. In all, 7,493 consumer samples were fabricated from the four project collections.

Table 16. Consumer samples fabricated by group, cut and ageing period

	PHASE 1 (Master Group 481)											PHASE 2 (Master Group 483)											TOTAL
	Phase 1 Kill 1					Phase 1 Kill 2						Phase 2 Kill 1					Phase 2 Kill 2						
	NMS	NMH	MS	MH	Msex	NMS	NMH	MS	MH	Msex		NMS	NMH	MS	MH	Msex	NMS	NMH	MS	MH	Msex		
Cut & Ageing	481.1	481.2	481.3	481.4	481.5	482.1	482.2	482.3	482.4	482.5	**TOTAL**	**483.1**	**483.2**	**483.3**	**483.4**	**483.5**	**484.1**	**484.2**	**484.3**	**484.4**	**484.5**	**TOTAL**	
CUB045																	52	27				**79**	**79**
7																	6	5				**11**	**11**
21																	8	3				**11**	**11**
35																	10	5				**12**	**15**
56																	28	14				**42**	**42**
CUB081																	**16**	**8**				**24**	**24**
7																	8	4				**12**	**12**
21																	8	4				**12**	**12**
EYE075	**117**	**112**	**76**	**77**	**77**	**48**	**48**	**48**	**48**	**48**	**699**	**72**	**72**	**72**	**72**	**72**	**119**	**96**	**66**	**69**	**72**	**782**	**1481**
7	40	40	28	28	27	16	16	16	16	16	**243**	24	24	24	24	24	24	24	22	23	24	**237**	**480**
21	66	64	40	42	45	24	16	20	20	20	**357**	24	24	24	24	24	39	32	22	23	24	**260**	**617**
28																	16	8				**24**	**24**
35																	16	8				**24**	**24**
42	11	8	8	7	5						**39**						1	2	3	4	2	**12**	**51**
44						2	6	4	3	5	**20**												**20**
49												6	6	6	6	6	2	1	4	3	4	**44**	**44**
56												6	6	6	6	6	17	11	3	3	3	**67**	**67**
63						3	5	4	4	4	**20**						2	1	3	3	4	**13**	**33**
70												6	6	6	6	6		4	3	3	3	**43**	**43**
77												6	6	6	6	6	1	2	3	4	5	**45**	**45**
84						3	5	4	5	3	**20**						1	3	3	3	3	**13**	**33**
OUT005	**154**	**157**	**84**	**83**	**88**	**64**	**64**	**64**	**64**	**64**	**886**	**72**	**72**	**72**	**72**	**72**	**120**	**96**	**66**	**69**	**72**	**783**	**1669**
7	52	53	28	28	28	23	20	23	21	21	**297**	24	24	24	24	24	24	24	22	23	24	**237**	**534**
21	90	95	45	46	48	31	22	25	27	27	**456**	24	24	24	24	24	40	32	22	23	24	**261**	**717**
28																	16	8				**24**	**24**
35																	16	8				**24**	**24**
42	12	9	11	9	12						**53**						1	1	4	3	3	**12**	**65**
44						5	5	7	4	6	**27**												**27**
49												6	6	6	6	6	1	3	3	4	3	**44**	**44**
56												6	6	6	6	6	17	11	3	3	3	**67**	**67**
63						2	9	5	6	5	**27**						2	1	3	3	4	**13**	**40**
70												6	6	6	6	6	1	3	3	3	3	**43**	**43**
77												6	6	6	6	6	1	3	3	3	4	**44**	**44**
84						3	8	4	6	5	**26**						1	2	3	4	4	**14**	**40**
OYS036	**80**	**80**	**56**	**56**	**54**	**48**	**48**	**48**	**48**	**48**	**566**	**48**	**48**	**48**	**48**	**48**	**80**	**64**	**44**	**46**	**48**	**522**	**1088**
7	40	40	28	28	27	16	16	16	16	16	**243**	24	24	24	24	24	24	24	22	23	24	**237**	**480**
21	40	40	28	28	27	24	16	20	20	20	**263**	24	24	24	24	24	40	32	22	23	24	**261**	**524**
44						3	5	5	4	3	**20**												**20**
56																	16	8				**24**	**24**
63						2	6	4	3	5	**20**												**20**
84						3	5	3	5	4	**20**												**20**
STR045	**161**	**160**	**112**	**112**	**108**	**64**	**64**	**64**	**64**	**60**	**969**	**96**	**92**	**96**	**96**	**96**	**128**	**110**	**88**	**92**	**96**	**986**	**1959**
7	63	63	56	56	50	32	32	32	32	30	**446**	48	46	48	48	48	26	35	44	46	48	**437**	**883**
21	84	79	56	56	58	24	16	20	20	19	**432**	24	23	24	24	24	16	21	22	23	24	**225**	**657**
35																	28	12				**36**	**40**
42	14	18									**32**						1	2	3	4	3	**13**	**45**
44						3	5	4	3	4	**19**												**19**
49												6	6	6	6	6	2	2	3	3	3	**43**	**43**
56												6	5	6	6	6	51	28	4	4	4	**120**	**120**
63						2	6	3	4	5	**20**						1	2	3	3	4	**13**	**33**
70												6	6	6	6	6	1	3	3	3	3	**43**	**43**
77												6	6	6	6	6	1	3	3	3	4	**44**	**44**
84						3	5	5	5	2	**20**						1	2	3	3	3	**12**	**32**
TDR062	**111**	**110**	**73**	**72**	**69**	**47**	**48**	**48**	**48**	**47**	**673**	**48**	**48**	**48**	**48**	**46**	**80**	**64**	**44**	**46**	**48**	**520**	**1193**
7	40	40	28	28	27	15	16	16	16	16	**242**	24	24	24	24	23	24	24	22	23	24	**236**	**478**
21	64	65	43	44	40	24	15	20	20	20	**355**	24	24	24	24	23	40	32	22	23	24	**260**	**615**
42	7	5	2		2						**16**												**16**
44						3	5	4	4	3	**19**												**19**
56																	16	8				**24**	**24**
63						3	6	5	4	3	**21**												**21**
84						2	6	3	4	5	**20**												**20**
Grand Total	**623**	**619**	**401**	**400**	**396**	**271**	**272**	**272**	**272**	**267**	**3793**	**336**	**332**	**336**	**336**	**334**	**595**	**465**	**308**	**322**	**336**	**3700**	**7493**

Note: NMS = Never Mixed Steers, NMH = Never Mixed Heifers, MS = Mixed Steers, MH = Mixed Heifers and MSex = Mixed Sex.

5.3 Gold Coast fabrication of export samples.

As noted in section 5.2 primal cuts from a selected 24 head from the final Phase 2 second kill were trucked to a Gold Coast facility rather than the UNE boning room to facilitate a dry ageing comparison including testing in Japan. The base stress trial 7 and 21 day aged samples for each of the standard 5 cuts were prepared following identical protocol to that described in 5.2 within an expanded fabrication that included bone in and dry aged cuts evaluated by Japanese consumers in Sapporo. A summary of the procedures relating to the Gold Coast fabrication follow.

1. Consumer sensory samples were fabricated from 24 cattle within the Phase 2, kill 2 Tasmanian saleyard trial to compare wet (conventional vacuum packed) and dry ageing with a related overlay to extend the comparison to Australian and Japanese consumers.
2. The 24 head, 16 steers and 8 heifers, were selected from the "never mixed" trial groups with further screening to include heavier carcases and normal pH.
3. The standard collection for all the saleyard trial (and King Island) carcases was to collect the striploin, tenderloin, outside flat, eye round and oyster blade from a single carcase side with both 7 and 21 day aged samples then prepared from each cut. The wet aged primals from all kills were all vacuum packed at the Tasmanian abattoir.
4. For the Gold Coast subset cuts were collected from both sides of the 24 head to provide sufficient product to provide the standard samples in addition to those needed to compare wet and dry ageing and Japanese and Australian consumers.
5. The wet and dry ageing comparisons were restricted to the *M.longissimus dorsi* (LD) muscle and included portions from both the cube roll and striploin primals. The cube roll was not collected for any trial carcases other than the 24.
6. All bar one variation of the dry aged product were dry aged "on the bone" with the wet aged control cuts aged in boneless form. To facilitate this a standing rib and bone in loin from one side were collected (even nos of left and right) from each of 24 carcases, with a standard boneless cube roll and striploin collected from the other side.
7. Treatments were applied to 5 LD muscle positions noted as A (anterior) and P (posterior) within the standing rib/cube roll (CUB045) and as A1, A2 and P3 within the bone in loin/striploin (STR045 anterior to posterior). The extreme posterior P4 striploin position was used as "link" as in the primary project components.
8. The bone in loins and standing ribs were placed in cartons within liners after attaching an ID label and transported to the Gold Coast premises where, after further labelling to define which position subsequent consumer samples were to be taken at 21, 28, 35 or 56 days, they were transferred to a specialised dry ageing chiller and left exposed to air on steel racks.
9. The matching vacuum packed wet aged cube roll and striploin primals and the tenderloins, outsides and oyster blades from the 24 selected carcases were also transported to the Gold Coast facility to ensure they remained eligible for export to Japan.
10. On arrival at the Gold Coast premisest the spinalis (CUB081) muscle was removed from each wet aged cube roll, glued, and fabricated into a consumer sample. The portions of the cube

rolls and striploins designated as 7 or 21 days wet aged were also fabricated into consumer samples to align with treatment of the saleyard product at UNE. Those sections designated as controls to the dry aged treatments were re-packed and aged as “blocks”.

11. MSA consumer samples were fabricated from the wet aged tenderloin, outside flat, eye round and oyster blade primals after arrival at the Gold Coast facility and then aged as consumer samples prior to freezing on designated ageing dates following standard practice as described in 5.2.
12. For the TDR062 and OYS036 two samples were prepared from each primal, four per carcase. The two samples allocated for Australian testing were aged for 7 and 21 days whereas the remaining two were aged 21 and 56 days and designated for Japanese testing.
13. Three samples were fabricated from each OUT005 and EYEO75 (6 per carcase) with a 21 and 56 day aged sample for Japan and 7, 21, 28 and 35 day aged samples tested in Australia.
14. There is a known reduction in MQ score with LD position from the CUB045 anterior to the STR045 P3. To avoid confounding treatment and position effects treatments were rotated across positions and sides as designated in Figure 32.

FINAL		CUB045		STR045		
		A	P	A1	A2	P3
1 (95) 237	L	AD5	JD5		J23	AD3
	R	AW5	JW5	A21	A7	AW3
2 (96) 239	L	JW5	A21	AW3	AW5	A7
	R	JD5		AD3	AD5	J23
3 (97) 240	**L**	J23		AD5	AD3	JD5
	R	**A21**	**A7**	AW5	AW3	JW5
4 (98) 241	L	AW3	A21	A7	JW5	AW5
	R	AD3		J23	JD5	AD5
5 (99) 242	L	JD5		AD3	AD5	J23
	R	JW5		AW3	A7	A21
6 (100) 273	L	A21	A7	AW5	AW3	JW5
	R	J23		AD5	AD3	JD5
7 (101) 274	L	A7	AW3	J23	AD5	JD5
	R		AD3	A21	AW5	JW5
8 (102) 314	L	JW5	AW5	A7	A21	AW3
	R	JD5	AD5		J23	AD3
9 (103) 315	L	A21	A7	AD5	AD3	JD5
	R	J23		AW5	AW3	JW5
10 (104) 316	L	AW3	A7	A21	JW5	AW5
	R	AD3		J23	JD5	AD5
11 (105) 317	L	AD5	JD5		J23	AD3
	R	AW5	JW5	A7	A21	AW3
12 (106) 318	L	JW5	A21	AW3	AW5	A7
	R	JD5		AD3	AD5	J23
13 (107) 319	L		AD3	J23	AD5	JD5
	R	A21	AW3	A7	AW5	JW5
14 (108) 351	L	JW5	AW5	A21	A7	AW3
	R	JD5	AD5		J23	AD3
15 (109) 352	L	JD5		AD3	AD5	J23
	R	JW5	A21	AW3	AW5	A7
16 (110) 353	L	A7		A21	AW3	JW5
	R	J23		AD5	AD3	JD5
17 (111) 251	L	AD5	JD5		J23	AD3
	R	AW5	JW5	A7	A21	AW3
18 (112) 267	L	JW5	A7	AW3	AW5	A21
	R	JD5		AD3	AD5	J23
19(113) 268	L	J23		AD5	AD3	JD5
	R	A7	A21	AW5	AW3	JW5
20 (114) 271	L	AW3	A21	A7	JW5	AW5
	R	AD3		J23	JD5	AD5
21 (115) 272	L		AD3	J23	AD5	JD5
	R	A7	AW3	A21	AW5	JW5
22 (116) 342	L	JW5	AW5	A7	A21	AW3
	R	JD5	AD5		J23	AD3
23 (117) 343	L	JD5		AD3	AD5	J23
	R	JW5	A7	AW3	AW5	A21
24 (118) 346	L	A21	A7	AW5	AW3	JW5
	R	J23		AD5	AD3	JD5

** red numbers are actual works body numbers
(xx) are tag numbers as used during collection.

Figure 32. Allocation of ageing treatment to 24 carcase CUB045 and STR045 utilised in dry ageing Japanese and Australian consumer study.

15. The treatments applied across the rib and loin (LD) were coded as:

    AD5 - Dry aged for 56 days on the bone and evaluated by Australian consumers.

    JD5 - Dry aged for 56 days on the bone and evaluated by Japanese consumers.

    J23 - Wet aged in boneless form for 21 days followed by 35 days of dry ageing (56 days in total) and evaluated by Japanese consumers.

    AD3 - Dry aged on the bone for 35 days and evaluated by Australian consumers.

    AW5 - Wet aged boneless for 56 days and evaluated by Australian consumers.

    JW5 - Wet aged boneless for 56 days and evaluated by Japanese consumers.

    AW3 - Wet aged boneless for 35 days and evaluated by Australian consumers.

    A21 - Wet aged boneless for 21 days and evaluated by Australian consumers.

    A7 - Wet aged boneless for 7 days and evaluated by Australian consumers.

    The A21 and A7 samples were utilised as the standard saleyard trial comparison.

16. The dry aged primals were taken from the dry ageing chiller at the designated ageing days, the portion designated for that date removed, boned, fabricated into consumer samples and frozen and any remaining portion with a later designated date returned to the dry ageing chiller.
17. The wet aged portions/blocks paired to the dry aged treatments were also fabricated into consumer samples and frozen on the same date.
18. After the last (56 day) samples had been fabricated and frozen those to be tested by Australian consumers were transported to UNE and incorporated into consumer picks.
19. The dry and wet aged rib and loin derived samples were consumer tested across 15 Australian consumer picks (groups of 60 untrained consumers as described further in section 6). The within animal comparisons were always tested within the same pick to provide a tight comparison.
20. Each Australian pick also included OUT005, EYE075, OYS036 and TDR062 samples with the standard 7 and 21 day ageing periods and the additional 28 and 35 day samples for the OUT005 and EYE075. Again all samples from a cut from any carcase were allocated to a common pick.
21. Samples allocated to testing in Japan were transported frozen to Sapporo and tested in conjunction with Japanese beef samples in Sapporo in early 2017.

6 Methodology – Consumer sensory testing

Standard MSA grill testing protocols (Gee et al., 2005) and summarised by Anon, 2008, were utilised for all project samples. Samples sourced from project carcases were evaluated in 187 consumer sensory "picks", each utilising 60 untrained Australian consumers. Consequently 11,220 Australian consumers evaluated project derived product. In addition further Australian dry and wet aged samples from the same carcases were evaluated in conjunction with Japanese beef by 540 Japanese consumers (9 picks) utilising identical protocols in Sapporo, Japan. The design, "posting" and cooking methodologies are summarised below.

6.1 Consumer sample allocation

MSA sensory protocols stipulate testing under a common protocol in which 42 samples are evaluated by 60 consumers within a pick. In a grill pick there are three sittings (sessions) of 20 people. Each consumer is served 7 samples, the first of which is a presumed mid eating quality "Link" to provide a uniform starting base. Link sample results are analysed separately to the subsequent 6 samples which provide the core trial comparisons. All samples are evaluated by 10 consumers.

The MSA sensory protocol requires that each consumer receive product expected to encompass a wide range of eating quality. To achieve this each pick is designed around the Link and 6 further products. There are 6 individual samples within each product resulting in 36 test and 6 link samples being evaluated in each pick.

In this project the products were defined by cuts with eye round (EYE075) and outside flat (OUT005) allocated to expected low eating quality product groups, tenderloin (TDR062) and spinalis (CUB081) allocated to the high end and striploin (STR045) and oyster blade (OYS036) intermediate. The link predominantly utilised the posterior (P4) striploin samples.

Once a suitable pick design was developed it was completed utilising MSA software incorporated within the AUSBlue database. The software provided a table as shown in Figure 32 with individual samples selected from those designated as "Available" within AUSBlue. The software assisted in selection by pre-sorting Available samples for the nominated cooking method with further automated sorts to group as desired for "picking". As a sample, defined by a unique Sequence number and EQSRef alphanumeric code, was picked the Sequence and EQSRef codes were placed in the selected product within the pick table as shown in Figure 33. The corresponding sample row in AUSBlue was then shaded yellow by the software, the status changed to "Picked" and a Taste Test Reference such as 1109/2/3 (Pick 1109/Product 2/Sample 3) added.

Ck=	GRL			Night=	1109			Status=	Tasted				
Item	Link Seq	Link ID	Prod1Seq	Prod 1 ID	Prod2Seq	Prod 2 ID	Prod3Seq	Prod 3 ID	Prod4Seq	Prod 4 ID	Prod5Seq	Prod 5 ID	Prod6Seq	Prod 6 ID
1	AUS80949	V87S	AUS84685	V3G2	AUS84920	H4H9	AUS85008	B0W4	AUS84689	Z8Y3	AUS84917	R2W4	AUS85000	C1W1
2	AUS80951	K80V	AUS84687	Y6N3	AUS84922	E4Y6	AUS84202	M5S4	AUS84071	S2Y4	AUS84164	K7U3	AUS84999	K5A2
3	AUS80955	W56F	AUS84070	U9X1	AUS85005	V3K8	AUS85007	W1K0	AUS84688	N0E0	AUS84916	Q5Y2	AUS84915	N8S8
4	AUS80957	W77T	AUS84686	B0H6	AUS84200	F4L8	AUS84923	J8G4	AUS84682	Z0S1	AUS85002	C1Z0	AUS84914	J6F5
5	AUS80961	A95Q	AUS84921	S9Z1	AUS84201	Y8F5	AUS84166	J1B0	AUS84069	S5C5	AUS84199	S7U6	AUS84684	L9M6
6	AUS80963	Z99D	AUS84165	V5M7	AUS85006	N0Y5	AUS84924	Q2G2	AUS84681	D6S0	AUS85001	U3V0	AUS84683	T8D3

Figure 33. Example pick table (Pick 1109) produced by AUSBlue software

To provide linkage between the 4 project collections (Two kills within both Phase 1 and 2) each pick included samples from at least two collections. Further, to strengthen ageing comparisons, both the 7 and 21 day aged samples from any cut were allocated to a common pick. All cuts from some carcases were allocated to the same pick and others spread across picks to again strengthen linkage and to enable any pick effect to be determined.

A typical pick design is shown in Figure 34 with the coloured shading indicating treatment.

Sample	LINK	PRODUCT 1	PRODUCT 2	PRODUCT 3	PRODUCT 4	PRODUCT 5	PRODUCT 6
1	STR045 (P4)	OUT005, SY1, NMH - 7	EYE075, SY1, NMH -7	EYE075, SY2, NMH - 7	STR045, SY2, NMH - 7	OYS036, SY1, NMH - 7	TDR062, SY1, NMH - 7
2	STR045 (P4)	OUT005, SY1, NMH - 21	EYE075, SY1, NMH - 21	EYE075, SY2, NMH - 21	STR045, SY2, NMH - 21	OYS036, SY1, NMH - 21	TDR062, SY1, NMH - 21
3	STR045 (P4)	OUT005, SY1, NMH - 42	EYE075, SY1, NMH - 49	EYE075, SY2, NMH - 42	STR045, SY2, NMH - 42	OYS036, K1, Msex - 7	TDR062, SY2, MS - 7
4	STR045 (P4)	OUT005, SY2, MS -7	EYE075, K1, NMS - 7	EYE075, SY2, MH - 7	STR045, K2, Msex - 7	OYS036, K1, Msex - 21	TDR062, SY2, MS - 21
5	STR045 (P4)	OUT005, SY2, MS -21	EYE075, K1, NMS - 21	EYE075, SY2, MH - 21	STR045, K2, Msex - 21	OYS036, SY2, NMH - 7	TDR062, SY2, MH - 7
6	STR045 (P4)	OUT005, SY2, MS -42	EYE075, K1, NMS - 21 Blk	EYE075, SY2, MH - 42	STR045, K2, Msex - 63	OYS036, SY2, NMH - 21	TDR062, SY2, MH - 21

Figure 34. Example of a representative consumer pick design utilised.

In Figure 34 the collection groups are K1 (King Island Phase 1 first kill), K2 (King Island Phase 1 second kill), SY1 (Tasmanian saleyards Phase 2 first kill) and SY2 (Saleyards Phase 2 second kill). The treatment codes are NMS (never mixed steers), NMH (never mixed heifers), MS (mixed steers), MH (mixed heifers) and Msex (mixed sex). As in this example the pick design included all ageing treatments (7, 21 etc) from any cut within the pick and a mix of collection groups and treatments. Cut was used to ensure the products (columns) represented an eating quality range benchmarked by outside flat (OUT005) and tenderloin (TDR062).

Each consumer was served one link sample followed by a sample from each of the 6 products. The order of serving was controlled by a 6 x 6 Latin Square as displayed in Figure 35.

LINK					
1	2	3	4	5	6
2	4	1	6	3	5
3	1	5	2	6	4
4	6	2	5	1	3
5	3	6	1	4	2
6	5	4	3	2	1

Figure 35. 6 x 6 Latin Square utilised in consumer product allocation by AUSBlue software

As shown in Figure 35 each product (designated 1 to 6) appeared once in each order position (row within column) and also once before and once after each other product. The software allocated product across 5 Latin Squares with each consumer allocated product samples in accordance with one column in the Latin square. This process ensured that potential halo or serving order effects were balanced out.

In accordance with the protocol consumers were served as pairs with the 5 steaks within any EQSRef designated sample halved after cooking and served to the allocated pairs. To further reduce potential interaction with consumer or serving order each of the 5 steaks within an EQSRef were allocated by software routine to a different serving order and to a consumer pair within each of 5 sub sets of 12 consumers. This resulted in any sample (EQSRef) being served in 5 of 6 possible serving orders and across a minimum of two sittings.

6.2 Physical allocation (Posting) of samples to control presentational order

To deliver the design criteria stipulated in the protocol the 5 individual steaks within each of the 42 EQSRef defined samples within a pick must be "posted". This process was again assisted by software incorporated within the AUSBlue database.

Once a pick design was entered into the pick table as described in section 5.1 a routine was triggered to "Publish" a "Pick Sheet". The pick sheet provided a list in Sequence order of the 42 samples in the pick including their EQSRef codes, taste test references and a check column. The pick sheets provided a check list from which to sort samples into designated picks and to confirm each was found prior to proceeding to posting. In practice, to provide efficiency, 20 or more picks were generally processed at one time with the 20 pick sheets amalgamated and the frozen samples sorted from UNE freezer storage into the individual picks at the one time. Styrofoam boxes labelled with the individual pick numbers and with plastic liners were laid out on tables in numeric order in proximity to a central position used for sample calling and allocation (picking). To maintain samples in a frozen state room temperature was maintained at 0 to 4˚C and new boxes of stored samples brought from the freezer immediately prior to picking.

A cross calling routine was employed at all points of the picking and later posting process to minimise the risk of error. One person called the sequence number on a sample label enabling a second person to look up the sequence from the amalgamated pick list and call back the EQSRef code using the phonetic alphabet. When the first person confirmed the EQSRef the second called the pick number and the sample was deposited within the nominated Styrofoam box.

Once the sort was completed each pick was re-checked against the individual pick sheet to confirm all samples were present. Any missing samples were either located by additional checking or substituted to complete the pick. Each pick was stored in a Styrofoam box and returned to the freezer at UNE after checking.

When confirmed a further software routine, "Convert Pick to Post", was enacted for each pick. This routine produced "Round Sheets", consumer plate and questionnaire labels and control files for use in subsequent posting, consumer serving and data entry procedures. An example of a round sheet is shown in Figure 36. The 21 round sheets for each pick (representing 7 rounds of cooking in each of 3 sessions of 20 consumers within a pick) were printed on A4 paper. Each sheet was then placed within a plastic sleeve to prevent contact with meat and then placed within a further 250 x 350mm vacuum pouch oriented to ensure it could be easily read with the open end to the right.

1109.1-5	*AUS84682*		*AUS84914*		*AUS84200*	1109.1-5
	Z0S1		**J6F5**		**F4L8**	
AUS85002		*AUS84686*		*AUS84923*		*AUS85007*
C1Z0		**B0H6**		**J8G4**		**W1K0**
1109.1-5	*AUS84688*		*AUS84915*		*AUS85005*	1109.1-5
	N0E0		**N8S8**		**V3K8**	

Figure 36. Example of a Round sheet utilised in posting consumer samples to control cook and serving order

The 1109.1-5 designation denotes Pick 1109, Session 1 (consumers 1 to 20) and Round 5, the 5th of 7 rounds with each consumer served one sample per round. There are 10 Sequence and related EQSRef codes printed on the round sheet is a 3 – 4 – 3 orientation.

Other than the first served link, where all consumers were served common samples, subsequent test samples from the 6 products, as detailed in section 5.1, required each sample to be served in a different 5 rounds, spread across sessions (with one in each subset of 12 people) and in 5 different serving orders (rounds). The posting process was utilised to deliver these requirements.

Each box of pick samples was brought from the freezer and the 42 sample bags sorted. After removing the 6 links the remaining 36 sample bags were sorted into EQSRef alphanumeric order and arranged in a 9 x 4 orientation on a stainless steel table located adjacent to a vacuum packer. Each sample bag was then opened by cutting across the upper end with the label clearly visible and checked to ensure each contained 5 steaks and that these were separated. Where they were frozen together they were broken apart.

One person (the caller) was positioned adjacent to the vac packer with a round sheet, within its vacuum pouch and inner plastic sleeve, attached to a metal clipboard. This person identified the EQSRef code at the right top corner of the round sheet and called the EQSREf. A second person (a picker) identified the bag with the corresponding EQSRef label and called back the sequence number to confirm the sample ID with their hand resting on the bag to maintain ID. When the cross check was confirmed one steak was removed from the sample bag and passed to the caller who placed it over the printed sequence and EQSRef on the round sheet.

The caller then called a second EQSRef, holding a finger on the code to ensure orientation, with the picker finding, calling the sequence number and then passing a second steak for posting over the second code. This was repeated 10 times for each round sheet after which the clip board was transferred carefully to the vacuum packer and the pouch vacuumed and sealed. This was repeated for all 21 round sheets with two complete bags utilised in each of the three link rounds.

The 21 completed round sheets were checked for leakers and then placed in two Styrofoam boxes with xxxx.1-1 on top and working through to the final xxxx.3-7 sheet at the bottom. The two stacks of rounds were separated by 3 further pouches of scrap meat designated as "Starters" utilised to stabilise grill plate temperature during cooking. Bubblewrap was then placed around the sheets to ensure that they would remain in orientation during transport even in the event of a "leaker" developing.

The foam boxes were labelled with the pick number and sessions, sealed and transported frozen to freezer storage in Dandenong, Victoria for subsequent consumer testing by Tastepoint, a contracted sensory testing organisation.

6.3 Sensory testing and consumer data management

All Melbourne consumer testing was conducted by Tastepoint Pty Ltd, a highly experienced sensory organisation. Consumer groups were reported using a charity based model in which common interest community groups were recruited with taste testing as a fund raising event. The group, rather than the individuals, were paid for participation. The groups provided basic demographic detail on potential consumers who were screened to include only people who preferred steak cooked to medium doneness, ate beef at least once per fortnight and were aged between 18 and 70 years old. The group model had been shown over time to deliver an excellent range of demographics.

Sensory venues, predominantly community facilities such as schools, sporting or church premises, with basic kitchen facilities and sufficient space to seat 20 consumers as five tables of four were identified and secured by Tastepoint. The subsequent sensory procedure followed MSA protocols summarised as follows.

1. The 21 round sheets for a pick were transferred from foam to a refrigerator at 4˚C twenty four hours prior to the scheduled sensory session to thaw.
2. Consumer label and output files for each pick, produced by the posting software, were emailed to Tastepoint. Consumer questionnaires were printed with a consumer ID and 7 sample (EQSRef) IDs printed on individual pages for each of the 7 samples allocated to that consumer. The 7 sample scoring sheets were preceded by 2 demographic sheets and followed by a further willingness to pay (WTP) sheet.
3. A further sheet of sample labels were printed onto Avery L7164, 64 to a page, label stock. An example portion of the label file is displayed in Figure 37.

GRL 1109.1 Tester ID number **1**	GRL 1109.1 Tester ID number **2**	GRL 1109.1 Tester ID number **3**	GRL 1109.1 Tester ID number **4**	GRL 1109.1 Tester ID number **5**
GRL 1109.1 Rnd 1 Tstr 1 **V87S**	GRL 1109.1 Rnd 1 Tstr 2 **V87S**	GRL 1109.1 Rnd 1 Tstr 3 **V87S**	GRL 1109.1 Rnd 1 Tstr 4 **V87S**	GRL 1109.1 Rnd 1 Tstr 5 **V87S**
GRL 1109.1 Rnd 2 Tstr 1 **V3G2**	GRL 1109.1 Rnd 2 Tstr 2 **V3G2**	GRL 1109.1 Rnd 2 Tstr 3 **H4H9**	GRL 1109.1 Rnd 2 Tstr 4 **H4H9**	GRL 1109.1 Rnd 2 Tstr 5 **B0W4**
GRL 1109.1 Rnd 3 Tstr 1 **E4Y6**	GRL 1109.1 Rnd 3 Tstr 2 **E4Y6**	GRL 1109.1 Rnd 3 Tstr 3 **S2Y4**	GRL 1109.1 Rnd 3 Tstr 4 **S2Y4**	GRL 1109.1 Rnd 3 Tstr 5 **Y6N3**
GRL 1109.1 Rnd 4 Tstr 1 **W1K0**	GRL 1109.1 Rnd 4 Tstr 2 **W1K0**	GRL 1109.1 Rnd 4 Tstr 3 **U9X1**	GRL 1109.1 Rnd 4 Tstr 4 **U9X1**	GRL 1109.1 Rnd 4 Tstr 5 **Q5Y2**
GRL 1109.1 Rnd 5 Tstr 1 **Z0S1**	GRL 1109.1 Rnd 5 Tstr 2 **Z0S1**	GRL 1109.1 Rnd 5 Tstr 3 **J6F5**	GRL 1109.1 Rnd 5 Tstr 4 **J6F5**	GRL 1109.1 Rnd 5 Tstr 5 **F4L8**
GRL 1109.1 Rnd 6 Tstr 1 **S7U6**	GRL 1109.1 Rnd 6 Tstr 2 **S7U6**	GRL 1109.1 Rnd 6 Tstr 3 **J1B0**	GRL 1109.1 Rnd 6 Tstr 4 **J1B0**	GRL 1109.1 Rnd 6 Tstr 5 **L9M6**
GRL 1109.1 Rnd 7 Tstr 1 **T8D3**	GRL 1109.1 Rnd 7 Tstr 2 **T8D3**	GRL 1109.1 Rnd 7 Tstr 3 **U3V0**	GRL 1109.1 Rnd 7 Tstr 4 **U3V0**	GRL 1109.1 Rnd 7 Tstr 5 **D6S0**

Figure 37. Example portion of consumer sample label file

The complete files included the 60 consumers in the pick with the 7 samples to be tested by each arranged vertically under each consumer number. It may be noted that the session (1109.1), round (1 to 7 vertically) and consumer (taster) number (1 to 5) appear on each plate label together with the EQSRef to direct the servers to the nominated consumers. It should also be noted that, in compliance with the protocol, a common Link EQSRef is served first to these 5 consumers (and also to consumers 6 to 10) and that the following samples are common for the consumer 1 and 2 pair and different for the consumer 3 and 4 pair.

4. The questionnaire sample labels were in identical order to each consumer column facilitating transfer of printed labels to the questionnaire forms should this be required in the event that the codes weren't printed directly utilising an input file. A second set of labels were printed with these transferred to 150mm diameter paper plates moving across the sheets from left to right working down with the plates stacked in a pile for each session. This resulted in the plates being arranged in consumer within round order within session to facilitate transfer to the serving trays at the venue.

5. The thawed round sheets were placed back in foam boxes and transported to the test venue together with equipment required. This included a large 3 phase Silex grill, associated power leads, two count up timers, cooking utensils, cutting boards, rubbish bins and liners, 420 pre-labelled paper plates, 60 collated questionnaire sets, 60 sets of plastic knives and forks, plastic cups, 12 fold up 1200 x 600mm tables, 20 chairs if required, table dividing partitions, and 42 plastic serving trays.

6. For the majority of venues where 3 phase power was not available a commercial generator was hired and towed to the venue.
7. At the venue a suitable cooking area was selected and the Silex grill positioned under a range hood or on a bench in an open external area as required to ensure all smoke was removed during cooking. Suitable space was required for trays of raw product to one side of the grill and for cooked product and trays plus a cutting board on the other. Three phase power was connected to the grill and the grill turned on and checked for temperature at least one hour prior to the session start time. A typical arrangement is shown in Figure 38.

Figure 38. Typical Silex grill arrangement for consumer test session.

8. As specified in the protocol a Silex S-163 clam shell grill with cast iron plates, the top grooved and the bottom flat, was used for all testing. The specified settings were:

   Height #3

   Weight #8

   Temperature 220˚C

   Top plate ratio #2.75

   ** It should be noted that other electronic Silex S-Tronix models may require adjustments to the settings to achieve a common result. Each grill must be calibrated prior to being used within a test session.

9. One bag of starters and the 7 round sheets for the first session were transferred to 8 plastic trays, within their vacuum sealed pouches, and placed on a bench adjacent to the grill to warm to room temperature. The tray order from the grill raw meat side was starters followed by rounds 1 to 7.
10. The plates for the first session were transferred to plastic serving trays with plates for consumers 17, 18, 19 and 20 round 7 placed on the first tray. A second tray for consumers 13, 14, 15 and 16 plates was placed on top of the first with this process continued until the top tray was round 1, consumers 1, 2, 3 and 4 placing the trays in the prescribed serving

order. The trays with labelled plates were then placed adjacent to the take off side of the grill.

11. Five pairs of 2 tables were erected and placed against each other to form five single 1200 x 1200 mm tables located within practical serving distance of the grill, typically in an open hall area. Corflute dividers were then erected on each table and secured with masking tape. Consumer numbers from 1 to 20 were then affixed to the dividers and a chair placed adjacent to each consumer booth. The arrangement of tables reflected the available facility but required sufficient space to enable easy serving and plate clearance. Figure 39 displays a typical consumer booth. Paired consumers were placed diagonally at each table to ensure sample numbers or scores could not be viewed or readily compared.

Figure 39. Typical arrangement of sensory testing booths.

12. A questionnaire, pen, plastic knife and fork, paper bread plate and plastic cup were placed in each consumer booth.
13. Each consumer was signed in on arrival at the venue and allocated a consumer number from 1 to 20. Their charity group was also recorded for payment purposes but no individual information that would allow personal identification was collected.
14. As the consumers started to arrive each of the thawed round sheet pouches were opened by cutting and folding back the upper bag surface to access the 10 steaks. The freezer wrap was removed from each steak and discarded. The correct sheet orientation was confirmed by ensuring the EQSRef codes were “right way up”.
15. When 20 consumers were seated the kitchen staff were notified. The cook lightly sprayed both grill plates with olive oil, placed the starter steaks on the grill dispersed across the plate and activated the two count up timers.
16. While waiting delivery of their first steak the seated consumers were instructed by the supervisor regarding the correct marking of the questionnaire demographic questions and product scoring.

17. All cooking processes and product serving was controlled by reference to the timers and the grill timing chart shown in Figure 39.The starters, shown as “Prelim. Scraps” on the chart, were removed at 05:00 minutes and discarded after checking that the degree of doneness was normal (medium). The chart dictated that each round of steaks be placed on the grill at a specific time (as shown in the “Load Next” column of the chart), that the upper lid be closed 45 seconds later (“Close Lid”) and the cooked steaks be removed 5 minutes post placement on the grill.

18. The placement of steaks on the grill and their subsequent removal followed a strict left to right, top to bottom order directly related to their 3 – 4 – 3 placement on the round sheet to retain ID through the cooking process. After the steaks were loaded the empty round sheet was transferred to a tray positioned to the cooked side of the grill and behind a cutting board.

Round No.	*Unload Steaks*	Load Next	*Close Lid*		Cut Up & Serve	
Prelim.		*START*	*00:30*			
Scraps	*05:00*	06:15	*07:00*			
1	*12:00*	13:15	*14:00*		15:00	
2	*19:00*	20:15	*21:00*		22:00	
3	*26:00*	27:15	*28:00*		29:00	
4	*33:00*	34:15	*35:00*		36:00	
5	*40:00*	41:15	*42:00*		43:00	
6	*47:00*	48:15	*49:00*		50:00	
7	*54:00*				57:00	*END*

Figure 40. Grill timing chart

19. At the nominated times the steaks were removed from the grill and placed on the cutting board in the same left to right, top to bottom sequence to maintain a constant cook time and ID.

20. In the time interval between removing one steak round and loading the next the grill plates were cleaned with a heavy metal scraper and wire brush. A light spray of olive oil was then applied to both plates prior to loading the next round.

21. As indicated by the timing chart, after loading each round the 10 steaks resting on the cutting board from the previous round were cut in half and transferred to the pre-labelled paper plates for serving. This resulted in a standard 3 minute resting time between cooking and serving.

22. A further ID check was conducted by the servers calling the EQSRef for each pair of plates on a tray and the cook confirming that EQSRef by reference to the empty round sheet behind the cutting board and related steak position on the cutting board . A round was completed

when both halves of the 10 original steaks were transferred to consumer plates for consumers 1 to 20, utilising the top 5 serving trays.

23. Servers carried each tray to the consumer seating area and located the correct 4 consumers by reference to the plate label and the matched consumer ID on the table partitions. Due to the system employed within the software and related procedure each table was served from a single tray each round. The servers also checked the EQSRef on the plate to ensure it matched that on the open questionnaire page.
24. The consumer area was managed by a senior staff member who ensured trays were delivered to the correct tables and directed removal of used plates, topping up of diluted apple juice and ensured that each consumer page was checked and marked prior to the next sample being delivered.
25. The standard consumer questionnaire sheets are included in the appendix as Attachment E for reference. In brief the first two pages recorded basic demographic data and were completed while awaiting the first sample to be served. There was a separate evaluation page for each of the 7 samples, identified by the EQSRef code printed in the top right hand corner. Each sheet had 100mm line scales for tenderness (anchored by not tender and very tender), juiciness (anchored by not juicy and very juicy), flavour and overall satisfaction (both anchored by Like extremely and dislike extremely), followed by a choice of four category boxes labelled unsatisfactory, good everyday, better than everyday or premium quality.
26. After delivery of the last sample consumers were asked to complete the willingness to pay (WTP) questionnaire page and thanked for their attendance. The WTP page presented 4 further line scales scaled from $0/kg to $80/kg with $10/kg increments with each line following the previous category box descriptions.
27. The process was repeated for the final two sessions with consumers 21 to 40 and 41 to 60.
28. All questionnaires were collected after each session and transported to Tastepoint premises for scanning and storage. Each questionnaire page was scanned utilising proprietary software which identified the marking on each line scale and calculated the score measured in mm from the left line end. This equated to a score between 0 and 100 as the line scales were 100mm long. Overall rankings and other questions recorded by marking of multiple boxes were also identified by the scanning program. Each software generated was accepted by a human operator to trigger the code to address further entries.
29. A checked csv file for each pick was then emailed to Rod Polkinghorne. Shortly after its receipt a software process within AUSBlue was activated to confirm the sample EQSRef codes and order for each consumer. After resolution of any inconsistencies relative to the original output file were resolved, further software functions completed file processing.
30. A consumer MQ4 score was firstly calculated by the software as (Tenderness * 0.3 + Juiciness * 0.1 + Flavour * 0.3 + overall * 0.3) for each of the 7 samples evaluated by each consumer. Each score from the 10 consumers that evaluated each sample (EQSRef) were then brought together and ranked from lowest to highest. An average score for all 10 consumers was then calculated and a further clipped MQ4 score calculated by averaging the central 6 scores after disregarding the lowest and highest two. A summary result table with

one line per EQSRef containing the average and clipped score for all line scales plus the calculated MQ4 and the category selection was produced and a manual check conducted on the product means.

31. A final software function within AUSBlue then transferred the sensory results for each EQSRef to the matching EQSRef row within AUSBlue completing the data available for that sample and changing the sample status to "Eaten". The source consumer file was stored to provide a base for individual consumer level data analysis including WTP and demographic detail.
32. Extracts of all project data were provided to Dr Garth Tarr, Dr Ray Watson and some Pathways Committee members for statistical analysis.
33. Further data extracts were prepared for Melbourne, Sydney and Murdoch University PhD candidates to combine with data on retinal scanning, infrared temperature and blood composition that they had collected within the project on farm and slaughter activity.

7 Results

Project primary statistical analysis was conducted by Dr Garth Tarr and Dr Ray Watson with further analysis by Dr Ian Lean and analysis of allied projects relating to blood chemistry by Kate Loudon, IRT camera data by Holly Cuthbertson and retinal camera related analysis by Maria Jorquera Chavez. Primary trial results are reported within the following sub headings.

7.1 Cattle utilised within farm, transport and slaughter groups

The number and distribution of cattle across farms, treatments within farms, vessels (for King Island), saleyards (for Tasmania) and abattoir rest period (overnight or 14 days) is displayed in Table 17. The numbers vary slightly from the design numbers due to two steers, one from King Island farm 1 and one from Tasmania farm 2 not being loaded due to temperament and welfare related reasons and due to a further unexplained steer death from Tasmania farm 1 and a heifer from Tasmania farm 4 being euthanased during the abattoir rest period. The second abattoir rest period corresponded with severe weather events including extreme cold temperatures and heavy rain.

Table 18 presents the mean, minimum and maximum values for primary King Island on farm measures being weight, crush score and flight speed measured 3 weeks prior to loading for despatch and the final weight and change from initial weight. It should be noted that these readings are all prior to the mixing, vessel allocation and abattoir rest treatments being applied as the cattle were selected through a sequential draft three weeks prior to shipment, eartagged to designate treatment group and then held as a single mob during the period between the initial handling and loading at which point they were drafted after weighing.

As only cattle from Vessel 2 were rested the vessel comparisons relate to all Vessel 1 and only the non rested portion transported on Vessel 2. Groups are coded as NMS (never mixed steers), NMH (never mixed heifers), MS (mixed steers), MH (mixed heifers) and Msex (Mixed sex).

Table 17. No of head by farm, treatment, vessel (King Island), saleyard (Tasmania) and rest period.

		Not Rested						Rested 14 Days						TOTAL HEAD
		NMS	NMH	MS	MH	Msex	TOTAL	NMS	NMH	MS	MH	Msex	TOTAL	
King Island	**Vessel**													
Farm 1	A	12		6		3	**21**						**0**	**21**
	B	8		8		3	**19**	**8**		**8**		**4**	**20**	**39**
Farm 2	A	12		6		3	**21**						**0**	**21**
	B	8		8		4	**20**	**8**		**8**		**4**	**20**	**40**
Farm 3	A		12		6	3	**21**						**0**	**21**
	B		8		8	4	**20**		**8**		**8**	**4**	**20**	**40**
Farm 4	A		12		6	3	**21**						**0**	**21**
	B		8		8	4	**20**		**8**		**8**	**4**	**20**	**40**
	Total Vessel A	24	24	12	12	12	**84**	**0**	**0**	**0**	**0**	**0**	**0**	**84**
	Total Vessel B	16	16	16	16	15	**79**	**16**	**16**	**16**	**16**	**16**	**80**	**159**
	Total King Island	**40**	**40**	**28**	**28**	**27**	**163**	**16**	**16**	**16**	**16**	**16**	**80**	**243**
Tasmania	**Direct/Saleyard**													
Farm 1	Direct	12					**12**	**12**					**12**	**24**
	Saleyard A			6		3	**9**			**5**		**3**	**8**	**17**
	Saleyard B			6		3	**9**			**6**		**3**	**9**	**18**
Farm 2	Direct	12					**12**	**12**					**12**	**24**
	Saleyard A			6		3	**9**			**5**		**3**	**8**	**17**
	Saleyard B			6		3	**9**			**6**		**3**	**9**	**18**
Farm 3	Direct		12				**12**		**12**				**12**	**24**
	Saleyard A				6	3	**9**				**6**	**3**	**9**	**18**
	Saleyard B				6	3	**9**				**6**	**3**	**9**	**18**
Farm 4	Direct		12				**12**		**12**				**12**	**24**
	Saleyard A				6	3	**9**				**6**	**3**	**9**	**18**
	Saleyard B				6	3	**9**				**5**	**3**	**8**	**17**
	Total Direct	24	24	0	0	0	**48**	**24**	**24**	**0**	**0**	**0**	**48**	**96**
	Total Saleyard A	0	0	12	12	12	**36**	**0**	**0**	**10**	**12**	**12**	**34**	**70**
	Total Saleyard B	0	0	12	12	12	**36**	**0**	**0**	**12**	**11**	**12**	**35**	**71**
	Total Tasmania	**24**	**24**	**24**	**24**	**24**	**120**	**24**	**24**	**22**	**23**	**24**	**117**	**237**
TOTAL PROJECT		**64**	**64**	**52**	**52**	**51**	**283**	**40**	**40**	**38**	**39**	**40**	**197**	**480**

Table 18. King Island On Farm measures prior to mixing and transport

GROUP	No	Crush Score (1 - 5)			Flight Speed (m/sec)			First Weight (kg)			Loadout Weight (Kg)			On Farm Weight Change		
	Head	Av	Min	Max	Av	Min	Max	Av	Min	Max	Av	Min	Max	Av	Min	Max
KING ISLAND - Processed on Arrival (not Rested)																
NMS	40	3.2	2	4	3.18	1.06	4.82	543	491	612	527	479	588	-16	-56	12
NMH	40	3.1	2	4	2.71	1.22	5.46	485	390	536	492	410	555	8	-13	40
MS	27	3.0	2	4	3.09	1.34	4.32	529	504	590	519	493	588	-10	-56	26
MH	28	3.0	2	4	2.85	1.65	4.48	482	390	545	493	405	585	10	-15	50
Msex	27	3.3	2	4	2.82	1.30	4.53	506	420	592	507	458	570	0	-36	90
ALL	**162**	**3.1**	**2**	**4**	**2.94**	**1.06**	**5.46**	**510**	**390**	**612**	**508**	**405**	**588**	**-2**	**-56**	**90**
Vessel 1	84	3.1	2	4	2.96	1.06	5.46	507	400	612	504	440	588	-3	-56	50
Vessel 2	79	3.2	2	4	2.91	1.30	4.43	507	390	600	506	405	588	-1	-42	90
KING ISLAND - Processed 14 daya after Arrival (Rested)																
NMS	16	2.9	2	4	3.02	1.98	4.47	548	497	622	534	479	590	-14	-38	8
NMH	16	3.1	2	4	2.67	1.45	5.47	483	435	530	488	449	532	5	-21	30
MS	16	3.4	2	4	3.03	1.79	4.27	536	489	598	522	476	594	-14	-36	2
MH	16	3.5	2	4	2.40	0.90	3.59	475	430	500	487	445	508	12	2	30
Msex	16	2.9	2	4	3.13	1.90	4.12	505	454	572	513	453	594	8	-24	82
ALL	**80**	**3.2**	**2**	**4**	**2.85**	**0.90**	**5.47**	**509**	**430**	**622**	**509**	**445**	**594**	**-1**	**-38**	**82**
ALL KING ISLAND																
ALL	**242**	**3.1**	**2**	**4**	**2.91**	**0.90**	**5.47**	**510**	**390**	**622**	**508**	**405**	**594**	**-1**	**-56**	**90**

Table 19 presents the summarised on farm data for the Tasmanian trial phase. As with Table 18 all measures are prior to shipment with all cattle held in a single mob on each farm between the initial handling and tagging to identify treatment group and the load out day for the saleyard consignments. The period between initial handling and despatch was two weeks for all Tasmanian groups in contrast to the 21 day interval for King Island (extended due to weather preventing sailing). The two phases also differed in that only the mixed treatments were transported to the two saleyards, this occurring at a common farm loading time. The NMS and NMH never mixed control groups were held on farm and despatched direct to the abattoir to arrive at a similar late afternoon the day prior to kill time as the saleyard mixed groups.

The data in Table 19 is that recorded for all cattle on the initial saleyard loading draft. The NMS and NMH remained on farm for a further day (the non-rested designation) or 15 days for the rested treatment.

Table 19. Tasmanian On-Farm measures prior to mixing and transport

GROUP	No	Crush Score (1 - 5)			Flight Speed (m/sec)			First Weight (kg)			Loadout Weight (Kg)			On Farm Weight Change		
	Head	Av	Min	Max	Av	Min	Max	Av	Min	Max	Av	Min	Max	Av	Min	Max
TASMANIA - Processed on Arrival (not Rested)																
NMS	24	2.5	1	4	2.53	1.13	3.82	531	414	676	544	410	674	12	-52	157
NMH	24	2.5	1	4	3.07	1.68	4.40	434	368	483	452	406	492	17	-20	51
MS	24	2.6	1	4	2.62	1.16	4.15	527	420	672	557	436	682	34	-46	185
MH	24	2.5	1	3	2.65	0.61	3.77	445	362	514	467	384	534	21	-5	65
Msex	24	2.5	1	4	2.48	1.00	4.71	491	400	672	508	417	716	17	-22	106
ALL	**120**	**2.5**	**1**	**4**	**2.67**	**0.61**	**4.71**	**486**	**362**	**676**	**505**	**384**	**716**	**20**	**-52**	**185**
TASMANIA - Processed 14 daya after Arrival (Rested)																
NMS	24	2.5	1	4	2.41	1.01	3.44	558	417	742	572	430	760	14	-68	84
NMH	24	2.3	1	4	2.88	1.66	5.18	435	400	465	457	419	504	22	-1	57
MS	22	2.4	1	3	2.20	0.90	4.67	552	393	690	578	410	718	30	-29	72
MH	23	2.7	2	4	3.01	1.61	4.14	438	374	528	453	390	506	15	-22	47
Msex	24	2.5	1	4	2.79	1.01	4.92	483	403	674	509	438	690	31	-3	116
ALL	**117**	**2.5**	**1**	**4**	**2.66**	**0.90**	**5.18**	**493**	**374**	**742**	**513**	**390**	**760**	**22**	**-68**	**116**
Saleyard 1	70	2.5	1	4	2.70	1.00	4.70	483	362	679	502	384	716	23	-46	112
Saleyard 2	71	2.5	1	4	2.57	0.61	4.92	495	393	690	519	410	718	26	-22	185
ALL TASMANIA																
ALL	**237**	**2.5**	**1**	**4**	**2.67**	**0.61**	**5.18**	**489**	**362**	**742**	**509**	**384**	**760**	**21**	**-68**	**185**

Table 19 display actual values recorded with some of the extreme liveweight differences highly improbable but retained without adjustment. These differences reflect practical challenges where farm scales and associated scale platforms and surrounds may be less than optimal.

From the data presented in Tables 18 and 19 it was concluded that the sequential draft methodology successfully produced randomised treatment groups of effectively similar composition and suitable for trial comparison purposes.

7.2 Statistical Analysis

To establish a form of relative animal effect for those used in the King Island project, denoted KIeq, Dr Watson fitted a model, MQ = animal + muscle*days aged to correct for muscle and days aged with the related analysis of variance (ANOVA) shown in Figure 41.

Analysis of Variance for mq

Source	DF	SS	MS	F	P
animal	242	52767.7	218.0	2.82	0.000
ms	4	473538.2	118384.5	1532.20	0.000
dagd	1	1726.0	1726.0	22.34	0.000
ms*dagd	4	1508.4	377.1	4.88	0.001
Error	2750	212476.8	77.3		

S = 8.790 R^2 = 71.76%

Figure 41. Analysis of variance for a model used to develop an "Animal Effect"

These animal effects were then used to fit a further model KIeq = pHu + sex*mixed sex + sex*rest with the resulting ANOVA displayed in Figure 42.

Analysis of Variance for KIeq

Source	DF	SS	MS	F	P
pHu	1	73.80	73.80	4.30	0.039
sex	1	0.66	0.66	0.04	0.845
rest	1	87.60	87.60	5.10	0.025
sex*rest	1	27.51	27.51	1.60	0.207
mix	1	70.00	70.00	4.08	0.045
sex*mix	1	10.86	10.86	0.63	0.427
msx	1	2.65	2.65	0.15	0.695
sex*msx	1	33.52	33.52	1.95	0.164
Error	234	4017.41	17.17		
Total	242	4339.48			

S = 4.143 R^2 = 7.42%

Figure 42. Analysis of variance of model fitted for Kieq

The ANOVA, shown in Figure 42, indicated that mixing and resting were significant in relation to KIeq together with pHu across all the King Island cattle. Further analysis was conducted for KIeq with a model including terms for treatment group, vessel and farm. This indicated that there was no statistical difference between farms or either of the two vessels.

The mixing and resting effects were similar for same sex and mixed sex groups being in the order or 1 to 2 MQ4 units.

The analysis of the Tasmanian saleyard Phase 2 data produced similar results in that there were no significant farm or sex group differences. Also in common with the Phase 1 data the mixing and resting effects were estimated at 1 to 2 MQ4 points.

ANOVAs for MQ also produced a very similar outcome for the sub set of mixed saleyard treatments as illustrated in Figure 43 and for the full Phase 2 data as shown in Figure 44. As is typical muscle, days aged, a muscle by days aged interaction, carcase weight and marbling were significant (P<0.05), with the very small range in ossification leading to non significance (all cattle being very young with the highest value 230).

The rest period is shown as significant (P>0.05) in both analyses but there was no significant difference between the two saleyards (Figure 43). The ANOVA in Figure 44 also displayed a significant (P>0.001) effect for muscle*position in line with MSA prediction model assumptions.

It should be noted that saleyard is confounded with mixing in this design as all non-mixed treatments were transported direct to the abattoir to align with commercial practice.

Analysis of Variance for mq (for "mixed" animals only)

Source	DF	SS	MS	F	P
ms	4	239909	59977	611.97	0.000
dagd	1	859	859	8.76	0.003
ms*dagd	4	1292	323	3.30	0.011
sy	1	16	16	0.16	0.688
rest	1	609	609	6.22	0.013
cwt	1	417	417	4.26	0.039
uoss	1	86	86	0.87	0.350
umb	1	1452	1452	14.81	0.000
pHux	1	928	928	9.47	0.002
wgn	1	106	106	1.08	0.298
Error	1371	134367	98		
Total	1387	378803	S = 9.89	R-Sq = 64.53%	

term	est	se
dagd=7	-0.7867	0.2657
sy=Po	-0.1087	0.2710
rest=0	-0.6764	0.2713

Figure 43. Analysis of variance for MQ relating to only mixed (saleyard cattle) from Phase 2.

Analysis of Variance for mq (for SY base data)

Source	DF	SS	MS	F	P
ms	4	145616	36404	382.12	0.000
dagd	1	1436	1436	15.07	0.000
ms*dagd	4	1653	413	4.34	0.002
pn	1	44	44	0.47	0.495
ms*pn	4	7081	1770	18.58	0.000
tts	5	1365	273	2.87	0.014
rest	1	912	912	9.57	0.002
cwt	1	25	25	0.26	0.608
uoss	1	99	99	1.04	0.308
cwt*uoss	1	134	134	1.41	0.236
umb	1	1059	1059	11.11	0.001
phu	1	1263	1263	13.26	0.000
hmp	1	3	3	0.03	0.867
rbf	1	1	1	0.01	0.907
wgn	1	11	11	0.12	0.733
Error	2197	209305	95		
Total	2225	591382	S = 9.76	R-Sq = 64.61%	

Figure 44. Analysis of variance for MQ relating to all Phase 2 cattle.

7.3 MSA grading and compliance

All cattle were graded by senior MSA graders in the early morning following each kill day. The primary MSA grading inputs are summarised for each kill in Table 20. There were obvious differences between each of the four kills.

The King Island kills displayed very similar mean scores for dentition, carcase weight, rib fat, hump, eye muscle area, ossification and fat colour as might be expected from sequentially drafted cattle from common mobs. Both mean AUS-MEAT and MSA marbling scores were reduced in the second kill in contrast to an increase in P8 fat.

The outstanding difference between the King Island kills however was observed in MSA compliance. While the percentage of rib fat failures increased from 1.8% in kill 1 to 5.0% in kill 2, pH compliance improved more than tenfold reducing from 39.3% in the first kill, directly off the vessels, to 3.8% after two weeks rest. Figure 44 illustrates the extreme pH distribution change observed after 14 days rest on good pasture. (Groups with a 481 prefix were processed directly after shipment in kill 1 whereas those with a 482 prefix were in kill 2 after a 14 day period on good pasture).

Table 20. Summary of MSA grading inputs and compliance rates by kill

		DENT	CarcWt	P8	HUMP	EMA	RiBFat	U-Oss	U-MB	A-MB	A-MC	A-FC	pH-U	LTemp
KING ISLAND Kill 1														
163 Head	Mean	0.1	270.2	6.8	56.3	68.9	6.6	168.1	361.0	1.2	4.0	2.0	5.7	5.4
	Min	0	206.8	5	30	50	2	120	200	0	2	1	5.43	4.4
	Max	4	322	10	75	94	14	230	740	5	6	4	6.72	7.7
	SD	0.5	20.5	1.1	8.2	8.2	2.5	28.2	68.7	0.7	1.1	0.7	0.2	0.7
	No MSA Fail						3				108		64	
	MSA Fail %						1.8%				66.3%		39.3%	
KING ISLAND Kill 2		**DENT**	**CarcWt**	**P8**	**HUMP**	**EMA**	**RiBFat**	**U-Oss**	**U-MB**	**A-MB**	**A-MC**	**A-FC**	**pH-U**	**LTemp**
80 Head	Mean	0.1	271.5	9.4	54.6	72.4	6.3	164.0	314.6	0.8	3.3	2.1	5.6	6.4
	Min	0	232.2	5	40	55	2	120	170	0	2	1	5.48	5.8
	Max	2	321.8	15	85	96	14	230	430	2	5	5	5.85	8.6
	SD	0.4	21.7	2.3	7.6	8.0	2.6	22.0	51.1	0.5	0.8	0.8	0.1	0.4
	No MSA Fail						4				36		3	
	MSA Fail %						5.0%				45.0%		3.8%	
TASMANIA Kill 1		**DENT**	**CarcWt**	**P8**	**HUMP**	**EMA**	**RiBFat**	**U-Oss**	**U-MB**	**A-MB**	**A-MC**	**A-FC**	**pH-U**	**LTemp**
120 Head	Mean	0.5	261.8	8.9	55.6	68.1	5.8	157.3	307.8	0.8	4.0	2.2	5.6	6.5
	Min	0	195.2	4	35	50	1	100	130	0	1C	0	5.41	5.7
	Max	2	397	20	80	99	17	230	520	3	7	4	6.65	8.8
	SD	0.8	53.3	2.8	10.3	9.8	3.2	25.0	75.8	0.7	0.8	0.7	0.2	0.5
	No MSA Fail						14				94		20	
	MSA Fail %						11.7%				78.3%		16.7%	
TASMANIA Kill 2		**DENT**	**CarcWt**	**P8**	**HUMP**	**EMA**	**RiBFat**	**U-Oss**	**U-MB**	**A-MB**	**A-MC**	**A-FC**	**pH-U**	**LTemp**
117 Head	Mean	0.7	268.2	6.2	55.1	69.1	5.6	158.2	292.6	0.6	4.1	2.5	5.6	7.0
	Min	0	188.2	2	40	52	1	110	160	0	2	1	5.43	5
	Max	4	425.8	10	85	140	21	230	460	2	6	5	6.28	9.3
	SD	1.1	56.8	1.6	10.4	10.6	3.5	25.9	63.2	0.6	0.9	0.7	0.1	0.9
	No MSA Fail						18				97		26	
	MSA Fail %						15.4%				82.9%		22.2%	

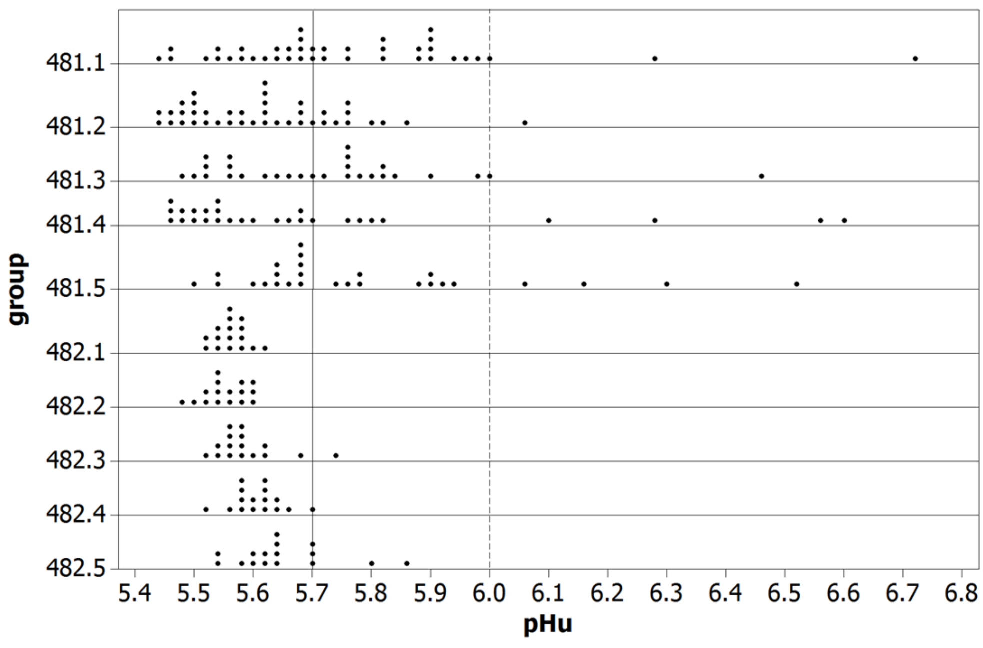


Figure 45. Distribution of ultimate pH within King Island treatment and resting groups

The Tasmanian kills provided a contrasting result with the second kill after 14 days rest having lower MSA compliance across all measures despite the mean scores for all MSA grading inputs being very similar other than P8 fat which was lower in kill 2. Low rib fat excluded 15.4% of kill 2 relative to 11.7% of kill 1 whereas pH failures removed 22.2% of kill 2 compared to 16.7% of kill 1.

When further divided into direct consignment and saleyard deliveries there are further differences as displayed in Table 21 for kill 1. It should be noted that all saleyard cattle were mixed at the saleyards in either same sex or mixed sex groups. There were no non-mixed cattle in the saleyard consignments, these being transported directly from the four properties of origin.

Table 21. Summary of MSA grade inputs and compliance for direct and saleyard consigned groups (Tasmania Kill 1)

TASMANIA Kill 1	Carcase No	SEX	DENT	CarcWt	P8	HUMP	EMA	RiBFat	U-Oss	U-MB	A-MB	A-MC	A-FC	pH-U	LTemp
	48 Head	Mean	0.42	260.49	8.94	54.27	67.21	6.21	157.92	308.13	0.77	3.94	2.23	5.61	6.61
Direct to Kill		Min	0.00	195.60	5.00	35.00	51.00	1.00	100.00	190.00	0.00	2.00	1.00	5.41	6.10
		Max	2.00	397.00	15.00	80.00	93.00	17.00	230.00	520.00	3.00	7.00	4.00	6.65	7.60
		SD	0.82	56.43	2.60	11.30	9.99	3.38	28.51	69.46	0.66	1.09	0.72	0.19	0.30
		No MSA Fail						4				33		6	
		MSA Fail %						8.3%				68.8%		12.5%	
TASMANIA Kill 1	Carcase No	SEX	DENT	CarcWt	P8	HUMP	EMA	RiBFat	U-Oss	U-MB	A-MB	A-MC	A-FC	pH-U	LTemp
	72 Head	Mean	0.5	262.7	8.9	56.5	68.8	5.5	156.8	307.6	0.8	4.0	2.1	5.6	6.4
Saleyard consignments		Min	0	195.2	4	40	50	1	110	130	0	3	0	5.41	5.7
		Max	2	390.2	20	80	99	14	230	480	3	6	3	6.22	8.8
		SD	0.9	51.5	3.0	9.6	9.7	3.1	22.6	80.1	0.8	0.6	0.7	0.1	0.6
		No MSA Fail						10				61		14	
		MSA Fail %						13.9%				84.7%		19.4%	

While the mean grade input scores were essentially identical the pH non compliance rates differed considerably increasing from 12.5% in the directly consigned cattle to 19.4% for the saleyard groups. Rib fat and meat colour changes displayed a similar pattern. A change of this magnitude would be of high commercial importance where MSA premiums are paid.

As shown in Table 20 the rest period was ineffective in Phase 2 of the project with compliance falling after the two week period. Table 22 summarises the data for the final saleyard kill after a 14 day rest subdivided as in Table 21 into saleyard consigned cattle and those held on the four farms over the same period. A comparison of Tables 21 and 22 reveals an increased pH non compliance of similar proportion for both direct and saleyard groups indicating a probable common cause believed to be the impact of a period of extreme wet and windy weather. Daily Bureau of Meteorology records for Smithton during the period are included in the Appendix.

The compliance margin between the direct delivered and saleyard consignments indicates that the relative difference between the two has remained. The comparison of pH distribution across the two Phase 2 kills is illustrated in Figure 46 with NMS and NMH being the never mixed steers and heifers respectively that were delivered direct from the four farms.

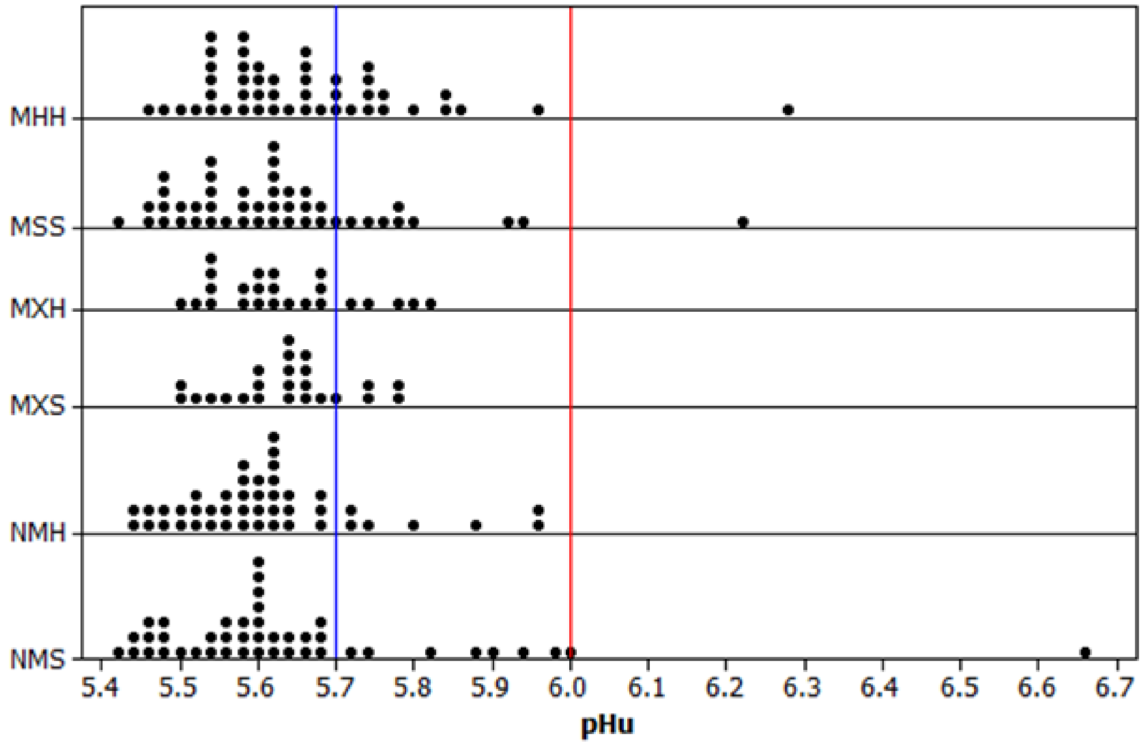


Figure 46. Distribution of ultimate pH for Phase two carcases

Table 22. Summary of MSA grade inputs and compliance for direct consigned and groups rested for 14 days after arrival from two saleyards (Tasmania Kill 2)

TASMANIA Kill 2	Carcase No	SEX	DENT	CarcWt	P8	HUMP	EMA	RiBFat	U-Oss	U-MB	A-MB	A-MC	A-FC	pH-U	LTemp
	48 Head	Mean	0.5	274.5	6.3	57.0	70.3	5.8	157.7	296.9	0.6	4.1	2.4	5.6	7.0
Direct to Kill		Min	0	217.2	3	40	53	1	110	160	0	2	1	5.43	5
	DIRECT	Max	2	425.8	10	85	140	21	200	460	2	6	4	5.99	9.3
		SD	0.9	60.6	1.8	12.1	13.0	4.0	24.5	66.8	0.6	1.0	0.7	0.1	1.1
		No MSA Fail						9				39		9	
		MSA Fail %						18.8%				81.3%		18.8%	

TASMANIA Kill 2	Carcase No	SEX	DENT	CarcWt	P8	HUMP	EMA	RiBFat	U-Oss	U-MB	A-MB	A-MC	A-FC	pH-U	LTemp
	69 Head	Mean	0.9	263.9	6.2	53.8	68.3	5.4	158.6	289.6	0.6	4.1	2.7	5.6	6.9
Saleyard consignments		Min	0	188.2	2	40	52	1	110	170	0	2	2	5.46	5.9
After 14 day Rest		Max	4	391.6	9	75	96	15	230	440	2	6	5	6.28	9
		SD	1.2	54.0	1.5	9.0	8.5	3.1	26.9	61.0	0.6	0.7	0.8	0.1	0.7
		No MSA Fail						9				58		17	
		MSA Fail %						13.0%				84.1%		24.6%	

Further investigation was pursued within the King Island groups to determine if the mixing treatments differed from the non-mixed. In the King Island component never mixed cattle groups could not be held on the farms of origin as they had to be shipped with the mixed treatment groups. They were maintained in their groups throughout the transport process including in abattoir lairage and during the 14 day rest period. Consequently any observed difference related to the mixing treatment rather than being confounded with any additional potential saleyard effect as in the Tasmanian Phase 2 study.

Tables 23 and 24 provide a comparison of the never mixed and mixed King Island treatment groups processed directly after arrival and after a two week rest period. As displayed there is a mean 7% pH related difference in MSA compliance in favour of the never mixed groups in the first kill, a similar result to that in Phase 2. Both categories of the King Island groups improved dramatically over the rest period with the never mixed groups retaining a mean 6% advantage and reaching 100% compliance after the rest period.

Table 23. Summary of MSA grade inputs and compliance for Never Mixed and Mixed groups during shipping from King Island (King Island kill 1 – not rested)

KING ISLAND Kill 1		DENT	CarcWt	P8	HUMP	EMA	RiBFat	U-Oss	U-MB	A-MB	A-MC	A-FC	pH-U	LTemp
	Mean	0.1	271.3	6.8	55.7	68.6	6.6	169.9	350.8	1.1	3.8	1.8	5.7	5.5
	Min	0	219.8	5	40	50	2	130	200	0	2	1	5.43	4.5
Never Mixed	Max	2	319	10	75	89	12	230	740	5	6	4	6.72	7.7
80 Head	SD	0.4	19.8	1.2	7.5	8.6	2.3	28.8	71.9	0.7	1.2	0.6	0.2	0.8
	No MSA Fail						1				50		29	
	MSA Fail %						1.2%				60.2%		34.9%	
KING ISLAND Kill 1		DENT	CarcWt	P8	HUMP	EMA	RiBFat	U-Oss	U-MB	A-MB	A-MC	A-FC	pH-U	LTemp
	Mean	0.1	269.3	6.8	56.9	69.1	6.6	166.4	370.8	1.2	4.1	2.1	5.7	5.3
	Min	0	206.8	5	30	55	2	120	270	0	2	1	5.45	4.4
Mixed	Max	4	322	9	75	94	14	230	630	4	6	4	6.6	6.8
83 Head	SD	0.5	21.1	1.1	8.7	7.8	2.8	27.7	64.2	0.7	1.1	0.7	0.3	0.7
	No MSA Fail						2				58		35	
	MSA Fail %						2.4%				69.9%		42.2%	

Table 24. Summary of MSA grade inputs and compliance for Never Mixed and Mixed groups during shipping from King Island (King Island kill 2 – Rested for 14 days)

KING ISLAND Kill 2		DENT	CarcWt	P8	HUMP	EMA	RiBFat	U-Oss	U-MB	A-MB	A-MC	A-FC	pH-U	LTemp
	Mean	0.1	276.3	9.0	53.9	73.7	6.0	160.9	325.0	0.8	3.2	2.0	5.6	6.4
Never Mixed	Min	0	241	6	40	55	3	130	220	0	2	1	5.48	6
32 Head	Max	2	321.8	15	70	96	14	190	430	2	4	5	5.61	6.9
	SD	0.5	21.0	2.1	6.9	8.1	2.7	18.2	45.7	0.4	0.8	0.9	0.0	0.2
	No MSA Fail						0				12		0	
	MSA Fail %						0.0%				37.5%		0.0%	
KING ISLAND Kill 2		DENT	CarcWt	P8	HUMP	EMA	RiBFat	U-Oss	U-MB	A-MB	A-MC	A-FC	pH-U	LTemp
	Mean	0.0	268.3	9.6	55.1	71.5	6.5	166.0	307.7	0.7	3.4	2.1	5.6	6.4
Mixed	Min	0	232.2	5	40	55	2	120	170	0	2	1	5.52	5.8
48 Head	Max	2	315	15	85	95	14	230	420	2	5	4	5.85	8.6
	SD	0.3	21.8	2.5	8.1	7.9	2.6	24.2	53.8	0.5	0.8	0.8	0.1	0.5
	No MSA Fail						4				24		3	
	MSA Fail %						8.3%				50.0%		6.3%	

The relatively tight and similar distribution of MSA model input traits is displayed in the Figure 47 box plots.

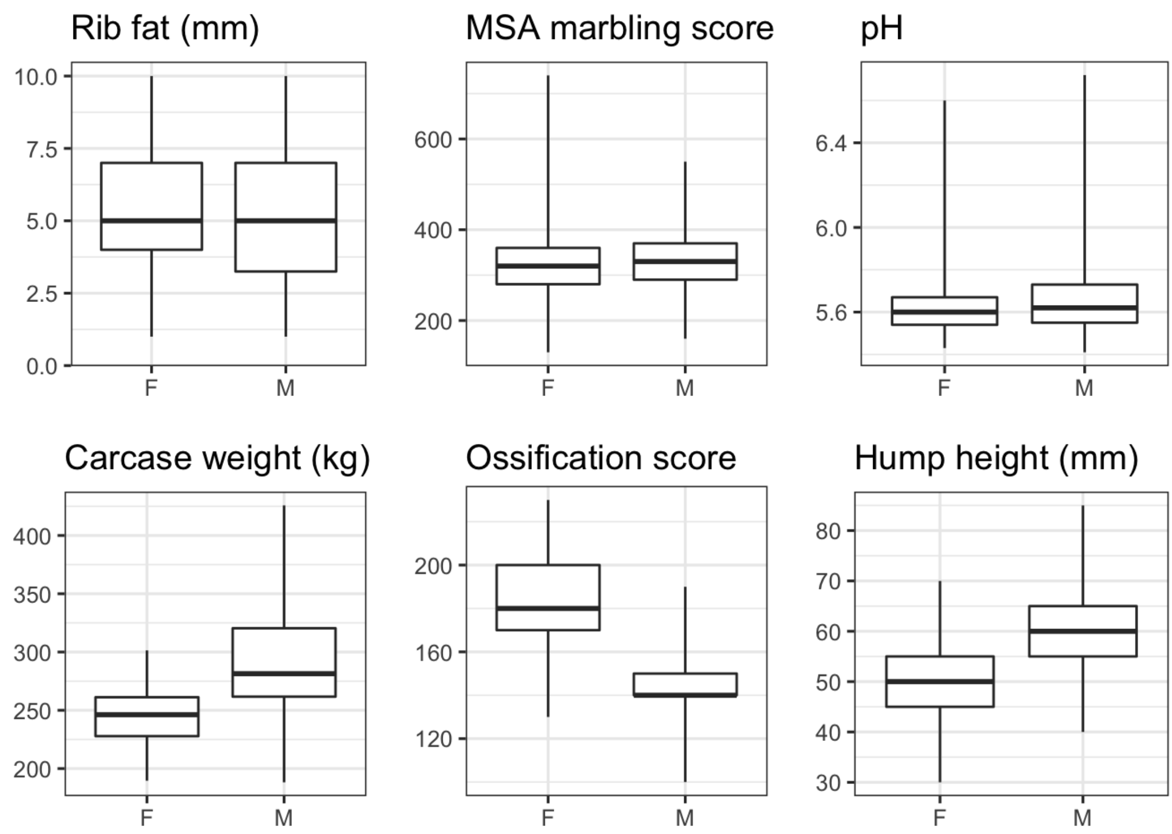


Figure 47. Distribution of MSA model input traits

In all kills the percentage of meat colour exceeding 3 was extremely high being 66.3% and 45% in the King Island kills and 78.3% and 82.9% respectively for the two Tasmanian kills. Meat colour had been recently removed as an MSA compliance factor due to earlier research reported in project L.EQT.0488 demonstrating it had no relationship to eating quality, differed between muscles and changed over the period from grading to retail display. These cattle provided an extreme example of difficulties encountered through meat colour at grading being inconsistent with observed pH.

7.4 Blood measures

Kate Loudon, a PhD candidate from Murdoch University, utilised the two project phases to investigate a wide range of blood measures with potential as either direct stress indicators or that could provide a better understanding of the impact of transport and mixing on physiological functions (Loudon, 2019a).

Blood was collected immediately after exsanguination from all cattle for subsequent laboratory analysis for selected biomarkers. These included plasma glucose, lactate, non-esterified fatty acids (NEFA), sodium, chloride, Beta hydroxybutyrate (BHB), magnesium, creatinine kinase (CK) and aspartate transaminase (AST). In addition muscle samples were taken from the *M.longissimus thoracis* and analysed for free glycogen, glucose and lactate.

While biomarkers were broadly similar between the two project phases CK differed in that it was approximately double in the first phase. This phase included a sea freight journey in addition to the mustering, loading, trucking and unloading procedures encountered in livestock marketing systems. This phase also found a highly significant beneficial impact on MSA non compliance though pH with a two week rest period in contrast with the second saleyard phase where no resting benefit was observed. The second phase resting period however was confounded by extreme weather and restricted feed availability relative to phase 1.

The selected markers were known to relate to various stress responses from dehydration, magnesium depletion, fasting, extreme exercise, fight and flight response and muscle damage.

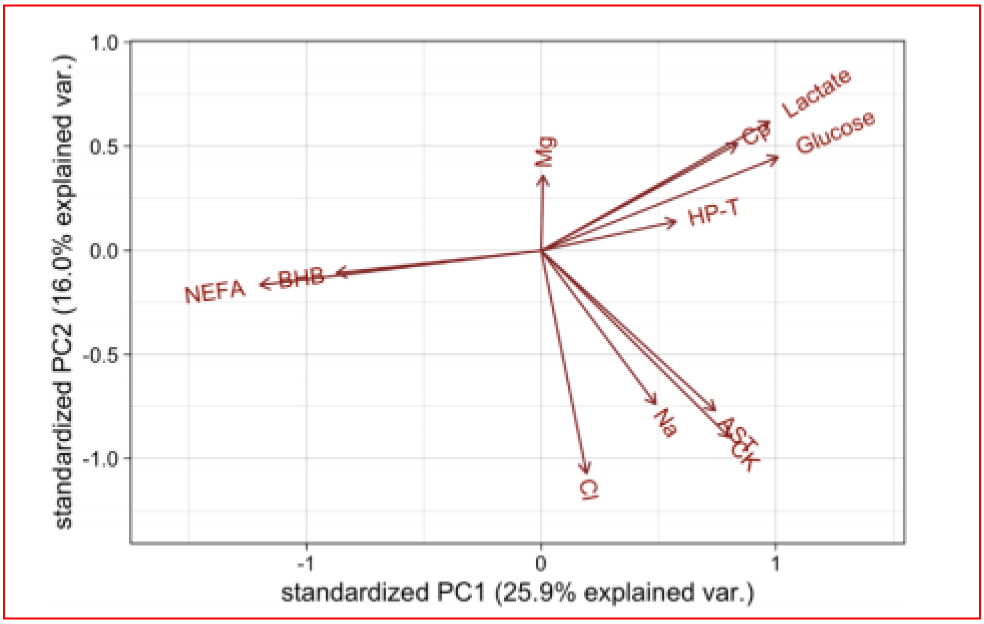


Figure 48. Loadings of principal component variables for biological markers (Loudon, 2019a)

Principle component analysis was utilised to group the various biomarkers as depicted in Fig.48 with the first two components accounting for 41.9% of variance.

The CK enzyme level was found to be the most correlated with meat quality, a valuable finding in that it indicates that muscle damage is an important contributor to stress prior to any visual bruising damage. The grouping of CK with AST is closely associated with muscle damage and vigorous exercise with Na and Cl indicators of dehydration, also a logical relationship to extended transport. Stimulation of fat and glycogen turnover and depletion together with feed deprivation are associated with changes in NEFA, BHB, Lactate, Glucose and CP. Magnesium is also associated with stress response.

A further important finding was that an effective two week rest period could overcome the detrimental impact of the marketing process.

The treatments imposed did not generate consistent effects on the markers studied which may reflect that greater variation between individual animal response than that measured across treatment groups.

The second paper (Loudon, 2019b) investigated direct relationships between the blood measures and eating quality. As stated in the abstract "Plasma glucose and L-lactate indicated a marked acute stress response at slaughter with a small detrimental impact on consumer score. The muscle damage enzyme markers creatine kinase (CK) and asparatate aminotransferase (AST) were strongly associated with a lower meat quality score (MQ4). Neither B-hydroxybutyrate (BHB) nor non esterified fatty acids (NEFA) were associated with MQ4, suggesting that fat mobilisation does not impact consumer sensory score.”

Differences were reported between muscles and a significant MQ4 measured improvement with 2 weeks rest pre slaughter.

Direct relationships between individual biochemical markers and MQ4 were not found although the associated physical effects related to changes in concentration were clearly related with the difference between muscles further indicating the need to include interactions in eating quality prediction. The data to date are not sufficiently clear to enable utilisation of an individual animal assessment to categorize cattle response or stress impact. While elevated CK could indicate higher risk animals pre slaughter the requirement to restrain and draw blood for analysis renders commercial application unlikely with current knowledge.

A rapid on line diagnostic test immediately post slaughter might be more commercially feasible and potentially useful as a prediction model input but requires more work to demonstrate individual animal effectiveness.

7.5 Infrared Thermography (IRT) measures

Holly Cuthbertson conducted the IRT measurement during both the on-farm and abattoir project components with the work contributing to her doctoral thesis at Sydney University. Work on this project followed earlier studies in which she had measured IRT and rectal temperatures utilising up to 4 alternative FLIR camera types in evaluation of University cattle and in a commercial abattoir.

Data from the earlier study is reported in Cuthbertson et.al (2019a) which contains important fundamental information in relation to methodology. The original data was not analysed prior to the King Island and Tasmanian projects but the subsequent analysis developed the techniques utilised in analysis of these.

Cuthbertson (2019a) provides a useful review of IRT background literature in addition to detail of protocol development. The orbital area of the eye was identified as a desired region for temperature measurement due to the high blood flow in the surface capillary vessels and the proximity to the brain making it highly responsive to any stress event that may affect temperature through blood flow. As changes in the capillary vessels can be recorded with high frequency video IRT it provides a means to measure heart rate in addition to temperature. This was utilised in the video IRT work conducted by Maria Chavez in her studies with Holly, by agreement, restricting her study to temperature alone.

In the IRT work reported the comparison is generally to rectal temperature. While rectal temperature is the traditional measure it is a single point and time record which might be expected to vary in relationship to the moving sequential measurements obtained by IRT where, depending on device, up to 1,000 readings can be obtained per second. The 4 cameras reported and also used in the present study ranged from 9 to 24 frames per second.

The IRT cameras record data from a large number of points dependant on the pixels per frame. For effective analysis a "region of interest (ROI)", in this case the eye, must be identified to firstly constrain analysis to a specific point, and then to elect which pixels are used for analysis and how. Practical problems arise in measuring animals in a commercial environment due to the speed required (60 per hour in these studies) and animal movement including moving forward and back within a race, blinking or head movement. Further the camera may re-focus at a critical time or record temperature of background material. The cameras are also very sensitive to environmental conditions including temperature and humidity.

Cuthbertson (2009a) produced methods to address these issues. Particular issues related to the recording time needed for accuracy and decision making relative to which temperature is utilised given the extensive number recorded. Her recommended method based on statistical analysis was to utilise a 1 second rolling median and the 97th or 99th quantile values due to their stronger correlation with rectal temperature. Longer recording periods did not improve accuracy and removal of peak temperatures through selecting the 97th or 99th quantiles reduced the risk of outlying values from external readings or movement.

Of concern for commercial application were differences between cameras from the same manufacturer and the variation with environmental conditions. For her study Holly manually identified the ROI by manual viewing of each frame to identify the actual animal and select frames that captured the eye. Given the many hours of records from 4 cameras on farm and in the abattoir and up to 64 frames per second this was an immense task and clearly not possible in a commercial environment where automated eye tracking software and real time analysis, crucially including outlier removal, is required.

A further issue that must be addressed prior to commercial deployment is the need for a positive animal ID during reading. As the IRT images relate to temperature only it is not possible to read any eartag within the field of view. For her work Holly utilised recorded kill order for identification with a second co-located conventional camera a possible approach for manual identification. Automation through reading of the RFID within NLIS tags provides a theoretical solution but would require some development to ensure correlation within a race where there is continual back and forward movement.

Cuthbertson et.al (2019b) report the on farm component of the projects. In all 481 animals were utilised from 8 farms, 4 on King Island and 4 in Tasmania. The King Island cattle were measured 3 weeks prior to shipping and the Tasmanian groups 2 weeks prior to transport.

A positive correlation was found between IRT and flight speed and, for differing groups and kill dates, pH, meat colour and post slaughter blood measures of CK, glucose, NEFA, Magnesium, FS and CS ($P<0.05$) but these relationships were not consistent.

The relationship between flight speed and IRT is interesting as are any relationships to slaughter results some weeks later as they indicate a potential to identify at risk cattle more susceptible to stress events. This could provide an on farm tool to utilise in culling or in careful management of farm to slaughter pathways including recovery rest treatments.

Challenges, and effectively a restriction on being able to adequately combine data for more powerful analysis, arose from the previously mentioned issues relating to differing environmental conditions during recording (particularly between the phase 1 and 2 components) and individual camera differences confounded by farm.

While a hypothesis that IRT could be a useful on farm tool is exciting these issues would need to be addressed for effective commercial utilisation.

The same 481 cattle were assessed again at the abattoir immediately prior to slaughter in two locations, unrestrained in the race leading to the knocking box and restrained in the knocking box immediately prior to and during knocking. These assessments are reported by Cuthbertson et.al (2019c).

Findings were not dissimilar to the on farm results (Cuthbertson 2019b) in that while significant correlations were found between IRT and glucose, lactate, NEFA, Mg, HAP, intramuscular fat, Cl, hump height, EMA and rib fat these correlations were not consistent across either locations (race vs. knocking box) or experiments (1 and 2).

Again, these results show promise but fall short of an immediately useable commercial tool. If successfully developed it would have great value in that a reading could conceptually be directly entered into the MSA prediction model and adjust MQ4 outcomes to reflect individual animal stress, possibly adjusted for muscle, and supplant current mandated delivery conditions. This would render all cattle eligible for MSA grading based on the grading outcome alone backed by recommendations for management prior to slaughter.

Cuthbertson et.al (2019d) further examined the potential linkage between IRT readings and consumer assessed eating quality as predicted by the MSA Prediction model. The MSA model has been developed utilising direct untrained consumer evaluation of beef samples over an extensive period, with the first version released in 2000. The statistical approach is used to firstly develop a statistic to best describe consumer response with weightings for tenderness, juiciness, flavour and overall satisfaction in relation to the weighted MQ4 (1 to 100) score and in turn to specify MQ4 bands that best align with categories of unsatisfactory, good everyday, better than everyday and premium quality (Watson et.al, 2008a, 2008b). All potential model inputs and their interactions are then related to the observed MQ4 for the considerable range of research projects undertaken and ultimately commercially applied through the prediction model.

In this project consumer scores were directly measured for five cuts with alternative ageing periods from all cattle slaughtered. This provided the means to examine the direct or additive effectiveness of including IRT as an additional prospective model input by comparing residual values of actual observed MQ4 relative to the predicted MQ4 values.

The abstract states that "Animals with elevated IRT were associated with decreased tenderness, overall liking, juiciness and MQ4 in some cases. The addition of IRT and cut interaction, particularly IRT measured at on-farm and at the knocking box, into the MSA model improved its predictive association with actual eating quality (base model Marginal R^2 = 0.650, IRT models Marginal R^2 = 0.692). These findings suggest IRT on-farm and the knocking box could facilitate more accurate prediction of eating quality".

This observation may be a little optimistic and likely to be challenged with alternative data sets given the somewhat erratic differences across the 4 kill groups, muscles and measurement locations but are encouraging none the less. The observed interaction between IRT and individual muscle MQ4, if substantiated by further work, is particularly pertinent to extension of MQ4 prediction across carcase muscles as it suggests that stress affects individual muscles differently, a core question in deriving effective eating quality estimates and supporting an hypothesis that "flight and fight" cuts, in this case the eye round and oyster blade, may be more reactive to stress.

Effective commercial application through inclusion in prediction modelling would be valuable both through improved accuracy and in particular if it could substitute for current mandatory best practise fixed requirements pre slaughter. Further industry value could potentially be gained if the on-farm reaction proved a useful predictor of response at slaughter as it could be used as an on farm measure for culling decisions or to group cattle for different management pre slaughter.

7.6 Video IRT measures

Maria Jorquera-Chavez, a PhD candidate from Melbourne University utilised animals within both project phases to examine relationships between heart rate, respiration rate and eye temperature relative to ultimate pH, meat colour and consumer sensory scores. For her work a thermal IRT (FLIR AX8) camera and an RGB (Raspberry Pi Module V2) video camera were mounted within a common enclosure to enable both IRT and visual animal views to be captured.

To overcome some of the issues identified by Cuthbertson (2019a) customised software was developed in MATLAB 2016A (Mathworks Inc, Matick, MA, USA) to located the head of animals within a race or knocking box and then identify regions of interest being the eye and nostrils.

Heart rate (HRT) was calculated from variation in blood flow within the eye capillary vessels together with eye temperature. Differences in temperature in the nostril area were used to calculate respiration rate (RR). These techniques had been previously utilised with human subjects utilising the Eulerian video magnification algorithm.

Difficulties with obtaining valid observation across a desired 10 second time period and with automated animal identification resulted in a lesser number of animals being successfully recorded at each of the two farm (race and crush) and abattoir (race and knocking box) locations with further reduction to animals with valid readings at multiple locations.

Jorquera-Chavez et.al (2018) report medium to high correlations between heart rate and cattle identified within high or low ultimate pH and meat colour groupings based on MSA compliance utilising knocking box readings from 120 of the King Island cattle. Increased heart rate was associated with increased pHu and meat colour but somewhat short of providing a consistent individual animal grouping. No significant relationship was found with eye temperature.

A further paper, Jorquera-Chavez et.al (2019), reported results from 68 of the Tasmanian trial cattle using knocking box camera data. In this paper HR, RR and temperature readings were moderately correlated with all sensory scales including MQ4. More recent (pers com) advice indicated that the highest correlations (Pearsons) was between farm and abattoir temperature readings.

8 Discussion

The significantly different results for a two week rest period after transport and prior to slaughter is a critical outcome for industry, establishing that while the rest period can be highly beneficial this is dependent on conditions, and requires positive management of food and shelter to be effective.

Further the significantly higher MSA compliance for never mixed groups is an important outcome and potentially critical where no resting period pre-slaughter is possible. While a positive 14 day rest period, as with the King Island phase, resulted in very large reduction in noncompliance the non-mixed groups maintained a superior level, as they did in the Tasmanian saleyard phase albeit the rest being ineffective.

The project provoked detailed scientific and statistical consideration in regard to categorising individual animal rather than treatment group stress levels. While the treatments were rigorously designed for, and were successful in, generating graduated additional stress challenge the reaction of individual animals within all treatment groups varied widely. Consequently response was not well categorised by comparing group means. No reliably effective means of identifying individual cattle of varying reaction was identified with options including blood metabolites, residual values from MSA model predictions, flight speed and behavioural measures and thermography all considered with none proving totally successful to this point. Further work is required to fully address this issue.

The type of stress is also likely to relate to the effectiveness of alternative measures. Chronic stress which can relate to periods of days, months or years is likely to be reflected by different measures to acute stress lasting from minutes to hours. Muscle pH at slaughter might be expected to reflect chronic stress, at least through the period between farm and slaughter, whereas IRT approaches seem likely to indicate more immediate reaction given the close relationship to the brain and blood flow measures. The association of CK with eating quality indicates that a large contributing cause of reduction relates to muscle damage despite a lack of visual bruising.

Conceptually the existing MSA pH measures combined with IRT could provide a useful suite of indicators across the stress spectrum with both incorporated as model inputs and applied differentially across muscles to improve eating quality prediction accuracy.

A clear individual animal indicator however remains elusive and was not identified through the project although there are encouraging indications from some IRT measures. Further work is required to better understand these technologies and to reduce the measurement variance through technology improvement. The variation in current systems is well documented by Cuthbertson et.al (2019a) and requires addressing prior to extensive commercial industry use.

9 Conclusions/recommendations

The project evaluated the impact on eating quality of stress created by transport, mixing and handling via two commercial supply chains, with related evaluation of management procedures to either reduce the impact or to recover prior to slaughter. The project incorporated a range of objective measures to assess their capacity to predict stress. These included crush score, flight speed, FLIR and retinal thermography used in conjunction with blood samples, pH and temperature declines recorded in conjunction with MSA grading.

The project successfully evaluated two complex commercial pathways which required detailed planning and rigorous execution. The project design enabled a varying degree of stress to be successfully imposed above pre-existing levels associated with cattle handling, loading, trucking and transit through a sea voyage or a saleyard environment. Extreme winter weather events experienced during the second phase of the project imposed additional pre-existing stress and may have overwhelmed the variation derived from the project interventions. Further studies with a milder level of pre-existing stress pressure could be valuable in confirming the eating quality impact.

The results confirmed a negative impact of mixing in saleyards and mixing during transport and in lairage on reduced MSA compliance and associated eating quality erosion. The direct delivery and non-mixed groups had substantially higher MSA compliance rates further reflected in moderately improved eating quality scores.

The rest period during the King Island phase was highly successful, dramatically increasing MSA compliance. In contrast, the second phase rest period was ineffective and accompanied by severe winter conditions that impacted both the cattle held at the abattoir and those retained on farm. This emphasised that to be effective recovery periods require active management with reference to prevailing conditions.

The treatments successfully generated graduated additional stress challenge, however the reaction of individual animals varied widely and it was found that animal response was not well categorised by comparing group means. No reliably effective means of identifying individual cattle with varying reaction was identified with options including blood metabolites, residual values from MSA model predictions, flight speed, behavioural measures and thermography all considered with none proving totally successful to this point. IRT readings at the knocking box and on farm did however show promise and, if substantiated and further developed for commercial application, would provide an attractive non invasive tool for individual animal stress assessment.

Further work is required to bring potential objective measures of stress to an acceptable commercial level. Success would enable more accurate eating quality estimates through including a stress indicator as a prediction model input. Identification of a reliable measure would simplify MSA supply preconditions as direct assessment of the effect could replace mandated best practice requirements. This would obviate the need to establish validated pathways that would render all cattle eligible for MSA grading. A substantial advantage of a direct individual animal measure would be the ability to factor in unique variables such as weather conditions or individual animal reactions. Hence there is strong interest in a single measure of stress that is practical, can be taken prior to slaughter and relate to any impact on eating quality as determined by consumer testing.

These projects provided an opportunity for collaboration between MSA staff, young researchers and PhD candidates from Murdoch, Melbourne, Sydney Universities and the University of New England (UNE). The involvement of young researchers in a major industry research project is regarded as an ideal principal to provide valuable experience and encourage mentoring and engagement.

10 Key messages

The project evaluated the impact on MSA compliance and eating quality of stress created by transport, mixing and handling using the two supply chains of shipping and saleyards.

The project included an evaluation of management procedures to either reduce the impact of stress or to recover prior to slaughter.

Direct delivery and non-mixed groups had substantially higher MSA compliance rates, further reflected in moderately improved eating quality scores across both the King Island and Tasmanian studies, this benefit being evident with or without a 14 day abattoir rest period.

A two week rest period for phase 1 cattle dramatically increased MSA compliance. However the second phase rest period, accompanied by severe winter conditions, proved less effective in terms of improving MSA compliance although direct delivered non-mixed groups maintained improved compliance relative to the mixed saleyard consignments both with and without a 14 day rest period.

This emphasised that to be effective recovery periods require active management with reference to prevailing conditions.

The reaction to stress of individual animals within treatment groups varied widely, however no reliably effective means of categorising individual cattle of varying reaction was identified.

A range of objective measures were utilised to assess their capacity to predict stress, including blood metabolites, residual values from MSA model predictions, flight speed and behavioural measures and thermography.

While some were promising, no objective measure was found to reliably predict individual animal stress level or its impact on eating quality. Further work is required to bring potential objective measures of stress to an acceptable commercial level.

Association of early on farm measures including flight speed and IRT technologies with subsequent slaughter and carcase results indicate that an on farm measure, even 3 weeks prior to shipment, may assist in identifying cattle more likely to be at risk of MSA non compliance or to have reduced eating quality. Such measures may also influence culling decisions.

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12 Appendix

12.1 Attachment A - Background Paper to MSA Pathways Committee

LDT Meeting, Sydney Qantas Club, Oct 17th 2011.

BACKGROUND

Objectives:

The meeting objective is to review the literature relating to the current exclusion of cattle from MSA, principally due to either saleyard movement or exceeding the time from farm despatch to slaughter. It is planned to review extensive research as a base and to consider recent studies of blood and urine measures.

If considered feasible a meeting outcome should be an agreed research framework that may allow an extension or refinement of the eligibility criteria.

An important element in this regard is the likely practicality of potential solutions within commercial supply chains as industry groups are keenly interested in potential outcomes.

MSA fundamentals:

The non-negotiable bottom line for MSA is that ***the consumer interest must remain paramount.*** To this end the degree of eating quality risk attached to any implementation decision must not detract from current levels. A change that may allow more cattle to be graded may be approved if the assessed risk of an inaccurate MQ prediction is consistent with the level of risk under existing arrangements. In the majority of circumstances however it is expected that changes will further improve prediction accuracy and reduce the risk of a lower than predicted result.

To date application of this principle has resulted in the exclusion of a large number of cattle under MSA with time from farm to slaughter a major issue. The current threshold of 36h is often exceeded due to both long transport distances and marketing via saleyards.

Industry is now requesting consideration of changes to allow “good cattle” currently excluded to be eligible for grading. There have also been a number of examples of the current exclusions being used as justification for alternative non MSA consumer identification systems.

The consensus view is that it would be preferable to have all cattle eligible for grading regardless of background and to modify the grading result appropriately. In short while large numbers may “fail” with the entire carcass or principal cuts being ungraded all would be eligible for the grading process.

Consequently there are two questions to consider:

1. Should the eligibility conditions be changed?
2. If so, what data is required to inform an expanded MQ prediction?

Current MSA screening criteria:

Currently to be eligible for MSA grading cattle are required to meet a number of pre-conditions:

6. Cattle must be consigned by a licensed MSA producer and accompanied by MSA and NVD documentation.
7. Cattle to be consigned must not be mixed within 14 days of despatch from the property of origin including during transport and within lairage prior to slaughter.
8. Cattle that are sick, due to calve or in oestrus are excluded.
9. Slaughter must occur by the day after property despatch.
10. Saleyard cattle can be eligible but must be sold through an MSA registered saleyard and meet conditions including no mixing and slaughter within 36 hours of property despatch. A 5 MQ4 point deduction is made to all model scores. Milk fed calves sold through a saleyard are not eligible.

These pre-conditions were established by the Pathways Committee to counter stress related conditions that were believed to be detrimental to eating quality but not able to be appraised during the grading process. It was recognised that excluded cattle could well include a proportion that exhibited acceptable eating quality but asserted that the variance and associated failure risk was necessary to provide a consumer guarantee.

In addition all cattle must comply with the Australian animal welfare standards and guidelines. Land transport of cattle (Anom 2008) which specify a maximum time of water deprivation (commencing when water is first withheld during mustering) of 48 hours for fit slaughter cattle. More restrictive provisions apply to bobby calves, cows close to calving and injured or sick stock. Cattle must be fed and watered as soon as possible after unloading. Where a 48 hour period is encountered a minimum rest period of 36 hours is mandated prior to any onward journey.

Current MSA prediction model operation:

Having met the screening provisions listed above the MSA prediction model then uses inputs relating to animal background, slaughter floor recorded data, traits measured on the chilled carcass and further traits classified by trained graders as inputs to calculating an MQ4 score for 40 carcass muscles within up to 8 cooking methods; a total of 143 scores. These scores are calculated at a constant 5 days post slaughter date and combined with individual muscle ageing factors, different for HGP and non HGP cattle, which allow further score modification when aged beyond 5 days.

Model inputs and brief comment on their application in prediction follows.

- Muscle and cook base. The most significant model input is a base table of 40 muscles, or muscle positions, by 1 to 8 cooking methods. The values indicate a "base animal" similar to the database mean. The base scores which range from 35 to 82 MQ4 points are further adjusted by grading inputs.

- % bos-indicus content. This is entered from the declared maximum on the MSA declaration. Analysis has established this as an important predictor with greater BI % reducing the scores. Data has determined that the effect varies widely between muscles with adjustments per % ranging from 0.03 to 0.136 MQ4 points.
- Hump height. This is a carcass measurement which can be electively used in lieu of a group declaration. It is also used as an over-riding cross check against declared values. Within the model the hump height is evaluated in relation to carcass weight to produce a *bos-indicus* % for calculation.
- HSCW. The carcass weight is an important data input taken from slaughter floor data, generally via the carcass ticket. It is used in prediction algorithims in conjunction with hump height to establish or confirm bos-indicus % for calculation and in conjunction with ossification, sex and muscle for direct MQ4 adjustment.
- Rinse Flush. If vascular infusion has been used the rib fat depth and carcass weight are reduced for MQ4 calculation in line with experimental data.
- HGP. The NVD provides the input of Yes or No for HGP use. Where an HGP has been used individual muscle scores are adjusted downward differentially with the calculation including weightings of 1, 1.5 or 2 reflecting available data for individual muscles. This corresponds to a maximum 16% MQ4 reduction. Ageing rate estimates are also adjusted with ageing slightly increased for implanted carcases.
- Sex. Currently M and F signify steer or female inputs which are used in conjunction with muscle directly and with ossification to adjust MQ4 estimates with M being the base default. A limited Northern Irish model version also includes B for bull although bulls are currently excluded from grading.
- MFV. Muscle specific adjustments from -4 to +6 MQ4 are applied for cattle advised as MFV (milk fed – weaned immediately prior to dispatch for slaughter) on the MSA declaration.
- Ossification. Ossification is determined by the grader evaluating the carcass backbone and ribs and scoring the degree of conversion from cartilage to bone against visual standards. The score, in 10's from 100 to 590, is used in conjunction with muscle, sex and HSCW to adjust the MQ4 prediction. Individual muscle multipliers range from 0.57 to 2.47 reflecting the relative impact of maturity on individual muscles.
- Rib Fat. Rib fat is measured in mm at the quartering site. Carcases with less than 3mm, or on which overall fat cover is deemed to be inadequate, are excluded from grading. Where fat is missing over a specific muscle this muscle may be rejected while allowing the balance of the carcass to grade. The rib fat effect is modeled as a constant for all muscles and in conjunction with marbling provides a fat content based MQ4 adjustment. The basis for the 3mm minimum was modeling work by MIRINZ that indicated an unacceptable gradient of temperature and pH conditions through major muscles where there was low or nil fat cover. The insulation effect of increasing fat rendered lower differentials from muscle surface to centre.
- Marbling. Marbling is assessed at the quartered surface of the LD against visual standards by the grader in 10's on a scale from 200 to 1,200. Individual muscle MQ4 adjustments range from 0 to 3.5 MQ4 points / 100 units.

- Hang. The base carcass represents AT (Achilles) carcass suspension. Direct MQ4 adjustments ranging from -5.2 to +8 are applied to each muscle for alternative hang inputs of TX (tenderstretch by the aitch bone), TL (tenderstretch by the ligament) and TC (tendercut).
- pH and temperature decline. A further screening requirement for abattoir MSA licensing is that slaughter floor electrical and chilling inputs are adjusted to achieve loin temperatures between 15°C and 35°C at pH6.0. This is established and monitored by assessing groups of 20 carcases periodically rather than testing every carcass. If these "abattoir window" conditions are not met carcases are excluded from MSA grading. The basis for this restriction is based on the scientific literature and MSA experimental data and aims to avoid cold shortening and heat toughening extremes while providing conditions for predictable post mortem ageing.
- Ultimate pH. A pHU measurement is taken from every carcass at grading. Any above 5.70 are rejected from MSA grades.
- Ageing. Individual ageing rates are applied to each muscle and interact with hang method, further adjusted for HGP. Adjustments range from 0 to 0.32 MQ4 points per day beyond 5 post slaughter. A standard pattern of linear increase to 21 days and curvilinear beyond 21 days constrained to a maximum of the 28 day linear equivalent is imposed.

Further Model Development:

The model has been progressively refined over 12 years on the basis of new data and the literature. To date the statistical approach has essentially been descriptive with the modeling process developed from the accumulated data. Experimental evidence has been considered by the Pathways Committee in conjunction with the literature and the model amended as appropriate.

Current work in progress is expected to allow addition of a bull sex category and an SS (Superstretch) hang alternative. Product from a large experiment involving extensive temperature at pH6 (T6) range in conjunction with gene marker, hang and ageing variations is currently being consumer tested and will yield substantial data that may allow model refinement including modified ageing estimates that include adjustments for T6.

Inclusion of gene markers is also under discussion with appropriate data being accumulated and there is some discussion of additional experimental work to evaluate potential differences between alternative HGP treatments. Preliminary evaluation of flavour chemistry in conjunction with consumer response is yielding interesting data and may improve understanding or prediction of flavour in conjunction with tenderness. How this would be incorporated into a grading system is still not clear.

Recent discussion has included the potential to use a form of biological model to assist in predicting muscle x cook effects that have not been adequately consumer tested using tested cells as a cross check. While the current model inputs have effectively been identified and quantified by study of the consumer result they naturally reflect underlying physical and biological mechanisms. Basic components such as the actual muscle and degree of restraint or stretching prior to rigor mortis are largely mechanical effects whereas the derived ageing estimates reflect more complex biological processes that are not directly viewed at grading but influenced by the pH and temperature decline and ultimate pH readings. Similarly ossification, sex and carcass weight inputs are indirect measures thought to be proxies for connective tissue content and solubility effects.

In considering the impact of pre slaughter stress from long distance transport, animal mixing and handling or prolonged time periods from farm to slaughter approaches can be considered that include direct measures, indirect estimators or underlying biological assumptions.

Under past practice inclusion/exclusion or adjustment of eligibility criteria would be addressed by product collection and consumer assessment. This is effective but requires a sufficient population and controlled and adequate range of alternative supply chains. What numbers would be required per cell, how many muscles would need to be tested, what are the potential interactions and which supply chain variations would need testing?

Alternatively, or more correctly in conjunction, what is our understanding of the biology and can we use this to develop direct or indirect indicators to apply as a precondition to or as part of the grading and prediction process? While much attention has been directed to ensuring adequate muscle glycogen levels at slaughter other mechanisms are also relevant. Inadequate glycogen will result in rejection on the basis or pHU>5.7. Does this eliminate all the low glycogen risk? Does it eliminate consumer risk? If so this becomes a simple grading application. Work by Warner et al. (2007), Gruber et al. (2010) and Ferguson et al. (2007, 2008) indicates that other mechanisms are involved and that while these are often difficult to detect by shear force they are detected by consumers. One potential mechanism appears to be lactate related. From the extensive blood and urine measures evaluated by Knowles and Wariss (2000), Ferguson et al. (2011), Pettiford et al. (2008) and others do we understand the biology sufficiently to explain consumer determined eating quality variance? What is the impact of hydration and do we have sufficient knowledge from Jacob et al. (2006a, 2006b) Pethick et al. (2009) and others to extend to consumer evaluated eating quality? What is the current view regarding electrolyte use in mediating losses or in recovery? What effective options exist for short term energy recovery pre slaughter?

It is anticipated that the meeting will need to discuss our current biological understanding in order to identify potential approaches to expanding MSA eligibility while maintaining or improving eating quality assurance to consumers.

The net result may include the following:

- Agreed expansion of base eligibility - for example a blanket extension to 48 hours from farm to slaughter.
- Certified extended movement protocols that prescribe rest and rehydration/feeding parameters within extended farm to slaughter movements.
- “Remediation” procedures that confer eligibility to cattle excluded due to prior handling.
- Additional grading inputs applied to all cattle graded.
- Additional grading inputs/criteria, for example blood or urine tests, mandated for cattle falling outside the standard conditions.
- Expanded eligibility for grading coupled with MQ4 discounts related to identified risks.
- Other changes to the prediction model.

Background R&D:

There is a considerable volume of prior work relating to animal transport, fasting and handling. The majority of this is welfare related with eating quality considerations less common although the two may be expected to be correlated. It is assumed that most meeting participants, having been involved in contributing studies over many years, will be familiar with the literature but a preliminary list of background papers follows, principally to identify useful additions. Can additional recommended papers please be advised/circulated prior to the meeting.

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Current work:

Work to be discussed at the meeting includes blood and urine analysis of multiple groups in NSW/QLD and WA. Drewe Ferguson and Alison Small will report on the outcomes of the NSW/QLD study. In brief this study involved blood and urine sampling of 510 cattle representing 45 different lots from 133 different vendors sourced direct and through saleyards. Distances to the abattoir ranged from 11 to 1941 km and time from farm despatch to slaughter from 16 to 259 hours. An unexpected feature of the data was the number of young cattle, many weaned onto trucks, that were accumulated and held for an extended period prior to slaughter. The cattle included some feedlot groups but were predominantly grass fed with pasture ranging from Mitchell and Flinders grass in western Queensland to highly improved pasture. Key laboratory measures were L-Lactate, β-hydroxybutyrate and urine specific gravity and osmolality. Most vendors were contacted by phone to discuss mustering, handling and loading. While of limited value for analysis this provided extensive background on industry practice and attitudes. A sub sample of the cattle were MSA graded and pH and temp declines were also recorded on some groups.

Dave Pethick and Peter McGilchrist will report on the WA analysis. In brief the work included two principal subsets of data. One involved 130 samples from cows trucked an extreme distance from the Kimberley to south of Perth and the other 294 feedlot cattle from two locations that had recorded flight speeds, MSA grading and either 2 or 5 hours transport to slaughter.

The extreme range of stock and conditions encompassed by these studies is expected to result in a wide range of laboratory readings; the question is the extent to which these may correlate or be useful in explaining stress and ultimately eating quality potential.

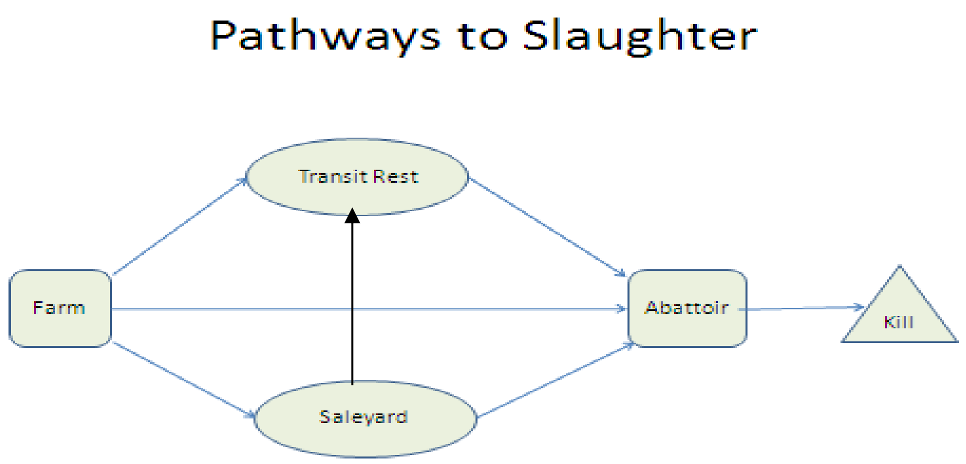
The meeting will need to consider any outcomes from these studies plus the background work in order to address potential responses to the industry request for increased eligibility.

Field Application:

Any proposed modification to the current MSA arrangements must be imposed on existing commercial practice. If the target of grading all cattle regardless of background is adopted, in contrast to the existing philosophy of excluding those with a known background risk, then considerably greater reliance is placed on grading model estimates which must be adjusted to counter the additional risk or enhanced by addition of accurate estimators that can characterise either the risk or, ideally, the direct impact.

A key objective of the current studies was to evaluate the potential for a direct measure of stress or implied eating quality risk. It was hypothesised that, if successful, cattle falling outside current eligibility criteria might be assessed on the basis of a blood or urine analysis which could render them eligible or confirm exclusion. If such a solution were considered plausible application would be relatively straightforward as “the test” could define risk/effect and the suppliers left to determine and control factors leading to failure or success without any need for MSA verification.

If this is not possible the onus moves to MSA to assess the direct eating quality risk and consider the degree of certainty relating to adherence to standards or notification of practices prior to delivery. The following diagram illustrates the principal routes to slaughter.



If we have no direct measure of stress/risk/EQ impact any move to broaden eligibility criteria without compromising consumer standards must either add conservative MQ penalties to model predictions to compensate for the estimated risk(s) or alternatively mandate a recovery mechanism. A possible example of this is an approved feeding and holding regime either at the abattoir or in intermediate facilities. The transit rest box in the diagram and possible direction via this from a saleyard movement represents this option.

MSA field interaction will occur at the following points:

- On farm. This relates to cattle preparation for shipment and loading. Pertinent issues include prior mixing of mobs, growth rate, nutritional regime, handling including mustering, feed and water conditions pre loading and loading practice with further possible guidelines regarding transport selection and criteria. At issue is the degree to which this is advice versus being mandated. If mandated the issue becomes monitoring compliance. The current structure for Producer Workshops and information releases has worked moderately well. Future extension activity, licensing and detailed mandatory practices and related compliance need to be developed in unison.
- Transport and rest. Guidelines or mandatory conditions are again pertinent questions. Available documentation is the NVD, on file at the abattoir, other state based movement documentation such as weighbills and potentially driver logbooks. Aside from hours of travel pertinent issues include low stress cattle handling within yards and crates including the use of prodders and dogs. Reliable audited processes are likely to be difficult but avenues for communication, extension and training in the transport sector could merit further pursuit. Contentious issues are likely to include recommendations regarding pre loading curfews.
- Saleyard operators. Ongoing communication with the sector is required building on past work and seeking further constructive adoption of approaches which reduce eating quality challenges. An obvious but difficult area of discussion is curfew with feed and water regimes for cattle held over also of interest.
- Remediation facilities. If future arrangements include specialised remediation facilities operated to restore compromised cattle to MSA eligible status these will be key contact

points. Direct communication and verified procedures should be feasible under likely structures.

- Abattoir lairage and slaughter. Existing arrangements provide a sound base able to cope with any additional MSA requirements.
- Grading. Again existing structures enable further development.

In general it appears that compliance issues are more readily addressed as the cattle move closer to the grading point. Graders operate at fewer locations and work within generally tight HACCP defined quality systems subject to regular stringent audit from multiple bodies. The plants are also more mindful of their direct product and brand reputation as opposed to a supplier who is furthest from the market and may have one head from thousands that are grouped within the brand.

Moderate scale remediation facilities would also be likely to be relatively few and subject to feedlot style QA and accreditation. MSA imposed standards may be incorporated within QA documentation and audited with relative ease compared to visiting all cattle properties.

Trucking practices and specific journey detail becomes progressively more difficult to certify other than via log book entries and more aligned to a code of practice approach supplemented by effective extension.

Farm level certification effectively relies on good will and a personal operator commitment to product quality due to the number and spread of properties. Detailed regular inspection or audit is impractical emphasising the need for effective extension to ensure a clear understanding of requirements and recommended practices and enthusiastic adoption.

Commercial application considerations:

At present there appear to be 3 principal areas of application that represent different commercial supply chains although affected by common biological mechanisms and hopefully solutions. The three are:

a) Genuine long distance road or rail transport.
b) Saleyard movements, often with short distances to sale and variable post sale distances. Animal mixing, multiple handlings and time constraints may often be more relevant than distance.
c) Sea transport from locations such as King, Flinders and Kangaroo islands.

a) Long distance transport:

An overview of distances and approximate travel times involved in Western Queensland, the primary area involved in long distance road and rail movements is provided in Table 1. While the majority of these cattle move to SE Queensland for slaughter there are also sizeable movements to the South (Naracoorte and Murray Bridge in SA). Other regions with similar distances and issues include central and Western Australia and the NT. It is assumed that data gathered in Queensland will be applicable in the other regions and vice versa.

Table 1. Distances and times to mid point of Dinmore and Beenleigh Qld.

Location	Google Km	Google Time	Google Time + 30%	Notes
Camooweal	2010	22.5	29.3	RT ok.
Mount Isa	1819	20.5	26.7	TD. RT ok. TY.
Cloncurry	1699	19.0	24.7	TD. RT ok. TY.
Hughendon	1427	17.5	22.8	TD. RT ok.
Clermont	983	11.5	15.0	TA. RT okay. RTY
Boulia	1713	19.5	25.4	TD. RT ok.
Winton	1351	15.5	20.2	RT ok. TY.
Longreach	1172	13.3	17.3	RT ok. RTY.
Alpha	1042	12.2	15.9	TD. RT limit(E).
Emerald	873	10.2	13.3	
Blackall	958	11.0	14.3	RT ok. RTY.
Windorah	1195	16.5	21.5	RT ok.
Quilpie	949	12.5	16.3	RT ok. RTY.
Charleville	739	8.75	11.4	RT ok.
Mitchell	558	6.75	8.8	RT limit.
Roma	471	5.75	7.5	
Dalby	204	2.75	3.6	
Thargomindah	997	13.5	17.6	RT ok.
Cunnamulla	800	10.0	13.0	RT ok.
St George	507	6.75	8.8	RT limit.
Goondiwindi	342	4.25	5.5	
Silverdale	73	1.0	1.3	TD.

TD. Tick clearing facility. TA. Tick infected zone.

RT okay. Type 2 road train. RT limit. Limit for type 2 road train.

RTY. Major trucking yard recommended by industry contacts. TY. Trucking yard.

From the theoretical times indicated it appears that transport times would rarely exceed 24 hours and in theory could meet the current “day after despatch” MSA rules. This is supported by data that suggests that of the 3.3 million cattle slaughtered in Queensland only 9% travelled greater than 1,000 km and 41% over 500 km in 2010. Actual total movement time however includes mustering, spelling or holding prior to loading, loading, any rest breaks en-route, unloading and lairage time.

Currently MSA specifies a “time of despatch” which coincides with the NVD Movement Commenced time. This is currently the only readily accessible reference time but any proposed change to MSA guidelines or regulations needs to consider any implications of mustering or other period prior.

Further implications for total property to slaughter intervals include driver hour restrictions, movement restrictions for type 2 road trains, tick infected and control zone regulations and animal welfare codes of practice. MSA approaches that work in concert with these issues may be more readily accepted and impose minimum additional effort.

National heavy vehicle regulations allow up to 12 hours work per 24 hour period for solo truck drivers. This includes mandatory rest breaks totaling at least 1 hour with work also including loading, unloading and vehicle servicing etc. An extension from 12 to 14 hours is possible under advanced fatigue management arrangements. With two drivers a truck can be essentially driven continuously for a day or so given that both drivers are coming off a mandatory rest break and alternate sleep in the sleeper cab during the journey. With careful planning relatively rapid transit times over long distances are therefore possible. The degree of difficulty in securing ideal driver conditions (prior rest for two drivers) is not known.

The Land Transport of Livestock code also allows a maximum of 48 hours off water for typical slaughter cattle which includes the period from first water deprivation. If water is withheld for 48 hours a minimum 36 hours of rest is mandated before any onward journey. Recommendations are for cattle to be fed and watered as soon as possible after unloading. Again this seems to provide an acceptable window for substantial movement, particularly as consideration of carcass weight losses is likely to encourage water and feed well within the maximum time frame.

More likely practical delays may arise from forced change of trucks or transit from infected or control cattle tick zones.

A substantial number of cattle from western Queensland commence their journey on type 2 road trains (6 decks on 3 trailers to a length of 53.5 metres). Road regulations however prohibit these vehicles from roads in the areas where major abattoirs and feedlots are located where B doubles (4 decks on 2 trailers to a length of 26 metres) are the largest units allowed. Type 1 road trains (4 decks on 2 full length trailers to a total of 36.5 metres) can access Toowoomba, Warwick and points west on most major roads but not the Brisbane area.

These truck restrictions dictate one of three responses at or before the point where road classifications change:

1. Offloading at a trucking yard and reloading on to separate trucks. This may or may not be arranged around water, feed and a rest period.
2. Cross-loading between trucks where cattle are run from crate to crate with the two trucks aligned parallel to each other. While this imposes a time delay it can be short and involves no water, feed or rest.
3. Re-arrangement of trailers with an extra prime mover so that two type 2 road trains (3 trailers x 2 decks) are converted to 3 B doubles (2 trailers x 2 decks). This is quicker than option 2 and does not involve cattle handling. The original trailers including turntables and lengths must however be suitable for use in B doubles. One trailer from the source type 2 road train must be shorter than allowed to meet B double length limits.

If a rest period is to be recommended as part of any potential protocol it would be efficient to arrange this to coincide with a required truck change and possibly driver rest break. The combination of road classifications and available trucking yards plus yard contractor reputation will be central to practical adoption. Interviews conducted under the blood and urine study indicated that the reputation of individual trucking yard contractors varied widely and were a factor in movement planning.

The use of trucking yards to rest cattle is extremely common in Queensland and a routine feature of cattle movement. There are widely held views, apparently based on anecdotal evidence but backed by considerable experience, that the rest periods enhance cattle welfare and improve subsequent performance as store cattle entering feedlot or grass fattening regimes or as slaughter cattle. A common view is that a minimum of 12 hours rest is required for best results with typical observations that the cattle on arrival will firstly lie down and rest for 4 – 6 hours, then drink and eat. Re-loading is reported as very difficult within 6 hours and easy after the common 12 hour spell.

All trucking yards offer unrestricted clean water and hay. The recommended contractors universally describe in depth their specification and sourcing policy for hay placing great store on the variety and harvest stage etc. Currently hay appears to be the only feed provided. Even assuming excellent quality it seems unlikely that energy concentration would be sufficient to build on animal glycogen reserves. To date this has encouraged MSA advice to move cattle direct to slaughter without breaking the journey as it was perceived that glycogen losses were a primary consideration. To build glycogen during the rest period would require a high energy feed sufficiently palatable to ensure intake by most cattle in a group. Given the location of principal trucking yards, their construction and the time needed for cattle to adjust to new diets there are limited practical options to boost energy intake.

Possible options canvassed for consideration are whole cottonseed, molasses or a standardised pellet. Major cotton growing areas and gins are located within reasonable distance of major centres and molasses is available from the coast. The feeding facilities needed for either are relatively basic and may be accommodated without extensive change to infrastructure. The addition of glycerine or electrolyte mixes to water may be a further consideration.

If more complex rations were deemed necessary transiting via a commercial feedlot may be warranted in order to access specialised feedmill and feeding equipment. Unfortunately the principal existing feedlots are mostly located beyond the reach of type 2 road trains which would require a further trucking change en-route for many cattle.

The issue of trucking cattle "straight through" to meet MSA day after despatch requirements versus extending the journey by a 12 hour rest break is of extreme interest to industry operators and requires formal objective study.

An allied research consideration for discussion is the impact of "non-glycogen mechanisms" that may impact on eating quality. While glycogen depletion is relatively understood MSA consideration of other mechanisms in regard to delivery standards has been limited to date. If a positive relationship was verified with transit rest it is likely to reflect other mechanisms which need to be examined. There is an argument that the existing exclusion of meat >5.7pHUltimate from MSA grades already deals with the low glycogen issue from a consumer perspective, placing the critical issue elsewhere in determining grade eligibility.

The major trucking yards were primarily established for rail movements and are located at major rail points. While some are owned by QNational all are operated by contractors and used for both road and rail movements. There are still a substantial number of cattle moved by rail each week including trains to Dinmore and less often to Beenleigh as well as Rockhampton and Townsville. The northern lines travel essentially east to the coast with Brisbane area consignments tracked south from Rockhampton or Townsville. Trucking yards are used en-route to inspect stock for welfare and to offload, feed, water and rest as required. While many observe that cattle "travel better" by train there is a substantial time increase versus road for most routes. Train capacity is also close to a practical limit due to track conflicts with coal. Scheduling is unlikely to allow additional cattle trains and extra wagons are restricted as train length is constrained by the need to enter sidings to allow coal trains to pass. Current trains have 44 decks carrying around 1,000 cattle.

While it would be interesting to compare rail and road impact on eating quality outcomes, and possibly necessary to complete an all encompassing MSA delivery protocol, the lack of ability to expand rail capacity at present renders this a lower priority. It is also important that as part of any proposed work that appropriate measures be included to quantify carcass weight loss associated with any of the treatments

Delivery arrangements are also impacted by cattle tick control regulations in northern and coastal areas of Queensland and NSW. Cattle from an infected zone are not allowed to move through a free zone without treatment and inspection at nominated points. Treatment is generally by plunge dip and where tick are detected a 5 day holding period is mandated prior to the second clearing dip. The location of tick zone borders, inspection points and abattoir locations influence routing and journey time in many instances.

Any potential to convert a 5 day imposed problem into an opportunity for a reduction in eating quality risk and to MSA eligibility is worthy of consideration. Some facilities such as Silverdale are routinely holding substantial numbers and the commercial aspects of instigating any recommended protocol are worthy of consideration.

b) Saleyards:

Saleyards remain a major marketing avenue for slaughter cattle, particularly in southern Australia. A high percentage of these cattle might be expected to grade well at the point of farm dispatch. Under MSA arrangements few saleyard groups meet MSA eligibility requirements which require continued segregation and slaughter within 36 hours of farm despatch. Constraints to eligibility relate to mixing of groups and total time from farm to slaughter.

A major study of saleyard movements conducted to assess biosecurity risk by the Australian Biosecurity CRC provides some useful background estimates. They report 6.2 million cattle being sold through 230 saleyards in 2006-07. These were sourced from 111,024 PIC's, 97.9% of which were properties other than feedlots. Movements from saleyards to slaughter accounted for 27.29% of stock sold.

The 95th percentile road distance of cattle received and despatched from saleyards was estimated at 445km with the 90th percentile at 274km and the 75th at 120km. The median distances travelled to sale (38.77km) was roughly half that travelled from sale (68.4km). In other words the majority travel relatively short distances. Road distances were estimated to be 1.2818 times the direct distance.
The density of cattle moving to saleyards also varied from a low of <0.001 per km^2 in central Australia to a high of 109 per km^2 in Gippsland, Victoria. From these estimates it would appear that distance is rarely the principal reason for saleyard cattle being unable to meet existing MSA time from farm to slaughter requirements.

Saleyard time constraints, raised as an issue by various groups, would appear to relate to the organisational time absorbed in coordinating movements, drafting, loading etc and in curfews both on farm and at the saleyard. Given the general literature consensus that nothing is gained from a welfare (Pethick et al. 2006), weight estimation (Smith et al. 1981) or transport (Ferguson et al. 2008) perspective some further recommendations regarding curfew practice in relation to MSA eligibility may be appropriate.

Saleyard movements were characterised into four types:

- Received group sold and remained in original group without additions (the current MSA stipulation). 2.62% of movements.
- Received group sold and kept together but aggregated with other group(s). 24.95% of movements.
- Received group sold and disaggregated into two or more complete groupings. 0.56% of movements.
- Received group sold and disaggregated into two or more groups with these then aggregated with other animals. 71.51% of movements.

From these estimates it would appear that the majority of saleyard movements involve the division of the original mob into several new mobs which are also grouped with other cattle. This factor is at odds with the current MSA requirements regarding no mixing of cattle within 14 days of slaughter.

The challenges relating to making more or all saleyard cattle eligible for grading consequently involve development of procedures that can provide eating quality surety post an environment of stock mixing and time delay. This in turn would seem to demand either new grading model inputs able to detect potential risk, sufficient MQ discounts to counter risk or proven post sale remediation procedures.

c) Sea freight:

While less common across Australia the number of slaughter cattle travelling by sea as part of the transit to slaughter is considerable. The sea journey is mostly relatively short but will vary in intensity with weather. The transport of cattle to and from the port also imposes 3 loading events with attendant stress potential. The cattle transported from King, Flinders and Kangaroo Islands are generally highly regarded and may be expected to grade well under MSA given that eligibility is maintained. It is assumed that conclusions reached from other research will be applicable to these movements although this may need to be confirmed by field validation.

12.2 Attachment B - Phase 1 - Detailed timetables

Timetable - Stress & Mixing Trial - King Island

Date	Action	Organisations	Order of Jobs to be Undertaken
21-Apr	King Island scoping visit		
27-Apr	Travel to King Island	PPL/MSA/Murdoch Uni	Muster cattle to yards - rest 1 hour
28-Apr	**Tag animals Farm 1 and 2**	/Syd Uni/Melb Uni	Fixed camera over the race recording cattle coming up the race to the crush
29-Apr	**Tag animals Farm 3 and 4**		Hand held camera recording the eye just after being caught in the crush before handling
	61 cattle each farm - Total 244		Tag animals/record/record weight - Obtain feed & water samples along with mob history
			Hand held camera takes second reading before releasing from crush
			Take Flight Speed & Crush Score
			Return cattle to designated paddock
21-May	Travel to King Island	PPL/MSA/Murdoch Uni	Brief participants
22-May	**Load cattle for trucking to Vessels**	/Syd Uni/Melb Uni	Muster cattle groups into yards and run quietly through crush.
	(simultaneously at all 4 farms)		Fixed camera over the race recording cattle coming up the race to the crush
			Confirm eartags are intact, replace as necessary, weigh.
			Hand held camera recording the eye just after being caught in the crush.
			Draft as facilities permit for subsequent loading.
			Load truck pens in accordance with eartag colour and schedule.
			Travel with trucks to wharf.
			Unload trucks (1 per farm) and re-pen on Statesman keeping groups separate. Load to designated pen by eartag colours.
			Observers to travel on the two vessels.Remaining personnel travel to Tasmania
			Observe unloading of Statesman at Stanley and ensure transfer of groups to trucks retaining separation.
	Cattle arrive at Abattoir from Statesman	PPL/MSA/Murdoch Uni	Manage unloading of trucks at Greenham lairage and ensure all groups are penned
			per plan in lairage or transferred to holding paddocks.
			(Some groups are boxed or sorted within this activity using eartag colour and detailed protocol).
23-May	Cattle arrive at Abattoir from Mersey	PPL/MSA/Murdoch Uni	Meet trucks off Mersey at Devonport and travel to Smithton.
23-May	**1st Kill - Greenhams Abattoir**	PPL/MSA/Murdoch Uni	Observe unloading at Greenham lairage, ensure groups are penned using eartag colour to control drafting or boxing as designated.
		/Syd Uni/Melb Uni	Group kill order to ensure groups are evenly distributed.
			Fixed camera over the race recording cattle coming up the race to the knocking box
			Hand held camera recording after head is caught in the knocking box just before stunning (10 secs)
			Record Animal ID to Kill number
			Take Bloods - cool - centrifuge
			Tag Carcasses
			pH declines
24-May	MSA Grading	PPL/MSA/Murdoch	Grade & tag Carcasses
	Boning/Cut Collection		Collect cuts at Boning & insert Primal ID tags
25-May	Ship to Greenhams Melb Depot		
27-May	Collect Depot Melb & Drive to Armidale	PPL	
29-May	Cut up samples at UNE	PPL/MSA/UNE	Cut up samples - Label - Pack - Chill
30-May	Cut up samples at UNE	PPL/MSA/UNE	Freeze according to Ageing
30-May	**Freeze down first (7 day aged) samples at UNE**		
6-Jun	Cattle already at Abattoir in holding paddocks	PPL/MSA/Murdoch Uni	Muster in rested cattle from abattoir paddocks
6-Jun	**2nd Kill - Greenhams Abattoir**	/Syd Uni/Melb Uni	Fixed camera over the race recording cattle coming up the race to the knocking box
			Hand held camera recording after head is caught in the knocking box just before stunning (10 secs)
			Record Animal ID to Kill number
			Take Bloods - cool - centrifuge
			pH declines
7-Jun	MSA Grading	PPL/MSA/Murdoch	Grade & tag Carcasses
	Boning/Cut Collection		Collect cuts at Boning & insert Primal ID tags
8-Jun	Ship to Greenhams Melb Depot		
9-Jun	Collect Depot Melb & Drive to Armidale	PPL	(Collect at earliest time cuts are available)
11-Jun	Cut up samples at UNE	PPL/MSA/UNE	Cut up samples - Label - Pack - Chill
12-Jun	Cut up samples at UNE	PPL/MSA/UNE	
13-Jun	**Freeze down 7 day aged samples**		Freeze according to Ageing

Timetable - KING ISLAND - Stress & Mixing Trial		
DATE	**ACTION**	
	Selection & Tagging of cattle on the 4 properties	
27-Apr	Travel to Melbourne - stay overnight	
28-Apr	Fly to King Island - Depart Melb (Essendon Airport) on SHARP Airlines SH922 departing 7:30am/Arr 8:15	
	Hire car 1 - Janine/Jess/Rod/Holly/Kate	
	Hire car 2 - Twin Cab Ute for generator, Hire car 3 - equipment	
9:30	**Farm 3**	
	Pictures Farm 3	
	8:30 Cattle mustered to yards - rest 1 hour	
	Set up for flight speed measure	
	Lay out tags	
	Fix camera over the race to record cattle coming up the race to the crush	
	Record cattle order	
	Hand held camera recording the eye just after being caught in the crush before handling	
	Tag animals/record	
	Record weight	
	Hand held camera takes second reading before releasing from crush	
	Take Flight Speed	
	Take crush score	
	Draft and Return cattle to designated paddock	
	Obtain feed & water samples along with mob history	
	Quick Lunch - Currie	
1:00pm	**Farm 2**	
	Pictures Farm 2 - no cover	
	12:30 Have 1st group of cattle mustered to yards - rest 1 hour	
	Set up generator (out of the yards)	
	Set up for flight speed measures (panels/barrells/hessian)	
	Lay out tags	
	Fix camera over the race to record cattle coming up the race to the crush	
	Record cattle order	
	Hand held camera recording the eye just after being caught in the crush before handling	
	Tag animals/record	
	Record weight	
	Hand held camera takes second reading before releasing from crush	
	Take Flight Speed	
	Take crush score	
	Draft and Return cattle to designated paddock	
	Obtain feed & water samples along with mob history	
5:00pm	**Accommodation - Currie**	
	Ph: (03) 6462 1288	
	8 rooms booked	
Friday 29/04/2016		
8:00	**Farm 3**	
	Cattle mustered to yards by 7:30	
	Pictures Farm 3	
	Take Lunch	
12:30	**Farm 4**	
	Cattle mustered into yards by midday	
	Pictures Farm 4	
Depart 4:15pm	**Drive to Airport - to arrive by 4:45pm**	
	Return Melbourne -SHARP Airlines SH927 departing KI at 5:30pm.	

12.3 Attachment C - Phase 1 - Resources and running sheets

To ensure all materials were available when required a resources list was prepared and all resources secured prior to the farm visits. Running sheets were distributed to parties involved to ensure no delays or miscommunication.

Resource requirements:

Cattle selection (Two Weeks Prior to Shipment)	
People:	Farm labour (2) to muster cattle and move through yards
	Recorder to record all data and control allocation of cattle to groups & eartag sequence.
	Thermographic and retinal camera operators (1 ex Melb Uni and 1 ex Sydney Uni)
	Person to eartag, read RFID
	Allow 2 days for a single team assuming 2 properties per day
	Require travel to King Island and return to Melbourne plus minimum of one nights accomodation on King Island.
	Require suitable vehicle(s).
Consumables:	Pre-written eartags plus spares, tag pen, 2 applicators and spare pins. Zee Tag Maxi 84 per farm
	Data lists and recording sheets
	Spare batteries and/or charger for cameras and readers.
Equipment:	RFID reader with storage and download capacity.
	Power outlet or generator if required for cameras.
	Retinal camera with thermographic and video output (Melb Uni).
	FLIR camera (Sydney Uni).
	Flight speed equipment.

Shipment Day - On Farms	
People:	One team per farm to allow loading in 2 hour timeframe (4 teams).
	Each team;
	Farm labour (2) to muster cattle, move through yards, draft to plan and load trucks.
	Recorder to record all data and control allocation of cattle to trucks and penning sequence.
	Thermographic and retinal camera operators (1 ex Melb Uni and 1 ex Sydney Uni)
	Travel to and from Melbourne for each participant.
	Require late afternoon travel to King Island and one nights accomodation.
	Require suitable vehicle(s).
	Afternoon travel to Burnie for some team members (3 to travel with cattle as shipboard observers) or return flight to Melbourne for any not involved with kill activity.
Consumables:	Colour spray mark for wither marking.
	Spare eartags and tag pen to replace any missing ID.
	Data lists and recording sheets
Trucks:	One 40 ft double deck per farm to transport 40 head to King Island wharf for loading on Vessel 2
	Two 40 ft double deck to transport 42 head to Devonport on Vessel 1 (Loaded sequentially at 4 farms).
Equipment:	Power outlet or generator if required for cameras.
	Retinal camera with thermographic and video output (Melb Uni).
	FLIR camera (Sydney Uni).

Shipment Day - At Wharf	
People:	Two people to manage unloading, drafting and ship loading protocol to travel with trucks from farm to wharf.
	Assistance from King Island stockmen experienced in loading ships and associated procedure.
	One observer to travel on each vessel.
	Require suitable vehicle or local travel arrangement.
Consumables:	Data lists and recording sheets.

Kill Day	
	Kill order list for lairage transfer.
	One person to control kill order & record time ex pen.
	One person to operate FLIR camera at knocking box.
	One person to operate retinal camera at knocking box.
	Recording sheets for eartag order.
	One person to record eartags.
	128 pre-numbered (kill order) tubes for blood, racks and Esky with ice.
	Two people to collect blood.
	128 pre-numbered (kill order) tubes for plasma, racks and Esky with ice.
	Centrifuge & extension lead if required.
	Two people to transfer blood to centrifuge and operate centrifuge.
	One person to take and record pH at scale.
	One person to take and record pH one hour post scale.

Grade, bone and collect cuts	
Chiller	Rib carcasses for MSA grading.
	Laminated carcase side tickets and stainless steel pins.
	Two people to attach carcase side tickets with pins to designate cuts to be collected.
	Two MSA graders.
	MSA grade all carcasses.
	Operators for hyperspectral, HunterLab, NIX and kuchida equipment and recording.
	Take hyperspectral reading of all carcasses.
	Take HunterLab reading of all carcasses.
	Take NIX reading of all carcasses.
	Take Kuchida camera reading of all carcasses.
Boning Room	Laminated pre-numbered Primal Tickets.
	Designated product codes established.
	Three people to place primal labels on cuts on slicing table.
	One person to oversee cut ID and ensure labels are attached to vacuum packing.
	One person to oversee cut retrieval and carton ID at packing. Cartons to be labelled prominantly.
	Reconciled list of cartons and cuts.
	MTC for cut transfer and despatch.

Running sheets - Cattle selection, identification and baseline measurement:

On the 28th and 29th April 2016 the project team travelled to King Island to select 61 cattle from each of the four farms in the trial, assign these cattle to treatments, identify them and gather the baseline measurements required before returning the selected 61 head to the paddock as a single mob.

The running sheets included below were distributed to the cattle suppliers prior to the visit two weeks prior to shipment.

INFORMATION FOR PARTICIPANTS - MSA STRESS & MIXING TRIAL	
Project Managers - Rod Polkinghorne	
Phone: 0410 300 905	
email:rod.polkinghorne@gmail.com	
Judy Philpott	
Phone: 0410 300 905	
email: judith.philpott@gmail.com	
ACTION	**ORDER OF JOBS TO BE UNDERTAKEN**
	TAG CATTLE ON EACH FARM
	Muster cattle to yards - rest 1 hour
	61 Cattle for the trial will be selected on Abattoir MSA spec as they come through the race. The group of 61 must be all one sex.
	Selected cattle are then tagged on both ears with coloured zeetags to enable correct drafting onto trucks 2 weeks later when they are shipped to the abattoir (tags supplied by Rod)
Farm 2 and Farm 4 - 28th April **Farms 3 and 4 - 29th April**	As they are being worked - a fixed camera over the race will be recording a body temperature map. Another hand held camera will take 2 measurements of the eye (just after catching & before releasing from the crush) to measure heart rate and temperature changes.
	These cameras will be set up an monitored by technicians from Sydney & Melbourne University
	The cattle are then weighed and recorded (Janine)
	As the cattle are released their flight speed & a crush score will be taken
	The mob of 61 is then returned to their paddock and kept as one mob for the next 2 weeks until trucked out
	Other information required - Feed & water samples along with the mob's history

As each of the four farms in the trial have a different cattle yard layout the implementation of the protocol was unique for each farm to achieve the desired result.

12.4 Attachment D - Phase 2 - Detailed timetable

Timetable - SALEYARD TRIAL - (Stress & Mixing Trial)			
Date	**Action**	**Organisations**	**Order of Jobs to be Undertaken**
7-Jun	Travel from Smithton to Farm 1	PPL/MSA/Murdoch Uni	Muster cattle to yards - rest 1 hour
8-Jun	Tag animals Farm 2 and 3	Syd Uni/Melb Uni	Fixed camera over the race recording cattle coming up the race to the crush
9-Jun	Tag animals Farm 4		Hand held camera recording the eye just after being caught in the crush before handling
	60 Cattle per farm - Total 240		Tag animals/record/record weight
			Hand held camera takes second reading before releasing from crush
			Take Flight Speed & crush score
			Return cattle to designated paddock
27-Jun	Load cattle for trucking to saleyards	PPL/MSA/Murdoch Uni	Muster cattle to yards
	(simultaneously at all 4 farms)	Syd Uni/Melb Uni	Fixed camera over the race recording cattle coming up the race to the crush
			Hand held camera recording the eye just after being caught in the crush before handling
			Draft according to designated saleyard
			Control cattle returned to 2 paddocks where possible.
			Supervise truck loading on farm
28-Jun	Saleyard Sale - Powranna	PPL/MSA/Murdoch	Trucks unload Powranna cattle at saleyard.
			Trucks continue to Quoiba saleyard after unloading at Powranna
			Powranna cattle penned in sale lane and experience mock sale environment.
			Powranna cattle held in designated groups post sale for collection on following day.
			Qoiba cattle held overnight prior to sale in designated groups under normal saleyard practice.
29-Jun	Saleyard Sale - Quoiba	PPL/MSA/Murdoch	Quoiba cattle penned in sale lane and experience mock sale environment.
	Cattle collected at Saleyards/Del to Greenhams	PPL/MSA/Murdoch	Oversight trucking & ensure groups are maintained.
	First Control Cattle collected from all 4 farms	PPL/MSA/Murdoch	Oversight trucking and manage to achieve similar arrival time to saleyard trucks.
			Observe unloading
30-Jun	1st Kill - Greenhams Abattoir	PPL/MSA/Murdoch Uni	Control kill order to ensure distribution per plan.
		Syd Uni/Melb Uni	Fixed camera over the race recording cattle coming up the race to the knocking box
			Hand held camera recording after head is caught in the knocking box just before stunning (10 secs)
			Record Animal ID to Kill number
			Take Bloods - cool - centrifuge
			pH declines
1-Jul	MSA Grading	PPL/MSA/Murdoch	Grade & tag Carcasses
	Boning/Cut Collection		Collect cuts at Boning & insert Primal ID tags
1-Jul	Ship to Greenhams Melb Depot		
4-Jul	Collect Depot Melb & Drive to Gold Coast	PPL	(Collect earlier or at Smithton if time can be reduced)
	Cut up dry aged control cuts at Top Cut	PPL	Cut up samples - Label - Pack - Chill - Freeze on designated dates
	Deliver domestic cuts to UNE Armidale	PPL	
6-Jul	Cut up samples at UNE		
7-Jul	Cut up & 1st Freeze down		Freeze according to Ageing
14-Jul	2nd group of controls collected from all 4 farms	PPL/MSA/Murdoch	Oversight trucking
			Observe unloading
14-Jul	Second Kill - Greenhams Abattoir	PPL/MSA/Murdoch Uni	Fixed camera over the race recording cattle coming up the race to the knocking box
		Syd Uni/Melb Uni	Hand held camera recording after head is caught in the knocking box just before stunning (10 secs)
			Record Animal ID to Kill number
			Take Bloods - cool - centrifuge
			pH declines
15-Jul	MSA Grading	PPL/MSA/Murdoch	Grade & tag Carcasses
	Boning/Cut Collection		Collect cuts at Boning & insert Primal ID tags
	Cut-up any USA designated cuts at Greenhams?	PPL/MSA/Murdoch	
	USA product exported to TTU	Greenham	
	Ship to Greenhams Melb Depot		
18-Jul	Collect Depot Melb & Drive to Armidale	PPL	(Collect earlier or at Smithton if time can be reduced)
20-Jul	Cut up	PPL	Cut up samples - Label - Pack - Chill
21-Jul	Cutup & Freeze down		Freeze according to Ageing
	Progressive cut-up of dry aged cuts at Top Cut		Cut up samples - Label - Pack - Freeze

12.5 Attachment E - Standard consumer Sensory testing questionnaire sheets

TPB TPB

Thank you for your participation today with our meat tasting

Our team is here to help you during your session and make this easy for you.

Before you start please listen to the instructions on how to use the scales contained in this questionnaire

Please use a black pen to fill in the form and where asked:

write crosses in boxes like this............ ☒

mark on the line scale like this... Not Juicy — Very Juicy

In between each sample please cleanse your palate by:

* first......... taking a sip of diluted apple juice
* then.......... chew a piece of bread
* and then..........take another sip of diluted apple juice

We are after YOUR opinion and therefore ask that you do not talk to anyone else in the room during the research session.

Now just a few questions about yourself (All this information is strictly confidential)

Date ☐☐☐☐☐☐

Your Group's Name ______________________

1. Please write in the boxes the postcode you normally live in ☐☐☐☐

2. Age Group: *(Use **X** in one box only)*

18,19 ☐ 20-25 ☐ 26-30 ☐ 31-39 ☐ 40-60 ☐ 61-70 ☐

3. Gender: *(Use **X** in one box only)*

Male ☐ Female ☐

4. What is the occupation of the main income earner in your household?:
*(Use **X** in one box only)*

☐ Manager
☐ Professionals (includes health professional etc.)
☐ Technicians and Trade Workers
☐ Community and Personal Services Workers
☐ Clerical and Administrative workers
☐ Sales Workers (includes retail sales etc.)
☐ Machinery operators and Drivers
☐ Labourers
☐ Home Duties
☐ Student
☐ Other

TPB TPB

TPB TPB

Please use a black pen to fill in the form and write crosses in boxes like this ☒

5. How often do you eat Beef?
(in any form such as steaks, roasts, stews, casseroles, kebabs, BBQ etc.?
*(Use **X** in one box only)*

- ☐ Daily
- ☐ 4-5 times a week
- ☐ 2-3 times a week
- ☐ Weekly
- ☐ Fortnightly
- ☐ Monthly
- ☐ Never eat beef

6.1. How many adults (18 and over) normally live in your household ?
*(Use **X** in one box only)*

- ☐ 1 Adult
- ☐ 2 Adults
- ☐ 3 Adults
- ☐ 4 Adults
- ☐ 5 Adults
- ☐ 6 Adults
- ☐ 7 Adults
- ☐ 8 and over adults

6.2 How many children under 18 years normally live in your household??
*(Use **X** in one box only)*

- ☐ 0 Children
- ☐ 1 Child
- ☐ 2 children
- ☐ 3 Children
- ☐ 4 Children
- ☐ 5 Children
- ☐ 6 Children
- ☐ 7 and over children

7 Please read the following statements
*and use **X** in one box only for the **one statement** that applies to you*

- ☐ I enjoy red meat. It's an important part of my diet
- ☐ I like red meat well enough. It's a regular part of my diet
- ☐ I do eat some red meat although, truthfully it wouldn't worry me if I didn't
- ☐ I rarely / never eat red meat

8. When you eat beef, such as steaks, what level of cooking do you prefer?
*(Use **X** in one box only)*

- ☐ Blue
- ☐ Rare
- ☐ Medium / Rare
- ☐ Medium
- ☐ Medium / Well done
- ☐ Well done

9. What level of income best categorises your combined household income ?
*(Use **X** in one box only)*

- ☐ Below $ 25,000 per year
- ☐ $ 25,001 - $$ 50,000 per year
- ☐ $ 50,000 - $ 75,000 per year
- ☐ $ 75,001 - $ 100,000 per year
- ☐ $ 100,000 - $ 125,000 per year
- ☐ $ 125,000 - $ 150,000 per year
- ☐ More than $ 150,000 per year
- ☐ Prefer not to say

10. What level of education have you reached? *(Use **X** in one box only for the highest level achieved)*

- ☐ Did not complete Secondary School
- ☐ Completed Secondary School
- ☐ A College/ TAFE course
- ☐ University Graduate

11. What is your cultural heritage ?
*(Use **X** in one box only)*

- ☐ Australian
- ☐ British descent
- ☐ European descent
- ☐ Asian descent
- ☐ Other
- ☐ Prefer not to say

TPB TPB

TPB TPB

All information collected in this survey is strictly confidential

PRODUCT:

Tenderness

Not Tender Very Tender

Juiciness

Not Juicy Very Juicy

Liking of Flavour

Dislike Extremely Like Extremely

Overall Liking

Dislike Extremely Like Extremely

Please mark **X** in one of the following boxes to rate the quality of the beef sample you have just eaten

Choose **one** only (you must make a choice)

- ☐ Unsatisfactory
- ☐ Good everyday quality
- ☐ Better than everyday quality
- ☐ Premium quality

TPB TPB

TPB TPB

Based on the beef you have just consumed:
Please mark the line at the price per Kg you believe best reflects the value for each category.

Unsatisfactory Quality

$0/kg $10/kg $20/kg $30/kg $40/kg $50/kg $60/kg $70/kg $80/kg

Good Everyday Quality

$0/kg $10/kg $20/kg $30/kg $40/kg $50/kg $60/kg $70/kg $80/kg

Better Than Everyday Quality

$0/kg $10/kg $20/kg $30/kg $40/kg $50/kg $60/kg $70/kg $80/kg

Premuim Quality

$0/kg $10/kg $20/kg $30/kg $40/kg $50/kg $60/kg $70/kg $80/kg

Are you the regular purchaser for your family ? *(Use* **X** *in one box only)*

☐ Yes
☐ No

TPB TPB

12.6 Attachment F - Smithton weather observations

Daily Weather Observations for Smithton, Tasmania for the period relating to abattoir activity during Phase 1 and 2.

Source: Bureau of Meteorology, Smithton Aerodrome station 091292

Activity	Date	Minimum temp (°C)	Maximum temp (°C)	Rainfall (mm)	Direction of maximum wind gust	Speed of maximum wind gust (km/h)	Time of maximum wind gust	9am Temp (°C)	9am relative humidity (%)	9am wind direction	9am wind speed (km/h)	9am MSL pressure (hPa)	3pm Temperature (°C)	3pm relative humidity (%)	3pm wind direction	3pm wind speed (km/h)	3pm MSL pressure (hPa)
Delivery of King Island cattle	22/05/2016	7.8	17.1	0	ENE	52	12:34	14.6	91	NE	28	1017	16.1	85	ENE	31	1012
First KI kill	23/05/2016	10.1	13.3	10.8	SW	76	14:37	10.1	90	WSW	37	1008.2	11.7	71	WSW	33	1011.8
KI rest period	24/05/2016	4.6	14.3	4	SW	43	13:24	10.7	88	WSW	20	1016.8	12.9	69	WSW	30	1016.4
	25/05/2016	1	15.8	0	NNW	39	19:54	8.2	98	SSW	7	1012.2	14.8	78	N	17	1006.5
	26/05/2016	8.1	13.1	4.4	SSW	35	14:17	9.3	93	S	9	1002.8	12.4	78	SSW	19	1001.6
	27/05/2016	2.6	13.5	0.2	SSW	31	12:36	6.5	98	SSW	11	1005.6	13.2	69	SW	22	1005.1
	28/05/2016	1.6	13.4	0.2	SW	44	11:45	5.4	96	SSW	7	1009.7	12	81	SW	26	1009.3
	29/05/2016	5.4	13.7	0.8	SW	43	13:53	9.3	94	WSW	7	1017.3	12.6	63	SW	28	1018.3
	30/05/2016	1.5	15.2	0	NE	26	13:15	6	89	S	11	1026.9	13.7	68	ENE	17	1026.8
	31/05/2016	3.8	14.4	0.6	ENE	43	12:05	10.2	86	ESE	19	1031.8	12.7	82	E	24	1029.9
	01/06/2016	8.7	14.4	2	E	39	11:21	10.3	81	E	19	1031	13.4	63	ENE	24	1029.1
	02/06/2016	3.6	15.2	0	ENE	24	12:33	7.7	94	ESE	9	1030.3	14.6	62	NE	13	1028.2
	03/06/2016	2.1	15.7	0	ENE	46	22:38	10.2	74	SE	9	1029.9	14.9	69	E	28	1027.1
	04/06/2016	10.2	16.7	0	ENE	65	23:38	14	74	E	30	1021.4	15.6	77	E	35	1016.8
	05/06/2016	14	15.6	15	E	67	02:20	15.6	94	NE	26	1005.1	14	96	NE	17	1002.9
Second KI kill	06/06/2016	12.2	14.8	23.8	WSW	20	05:49	13	96	WSW	9	998.9	13.5	88	SW	15	998.7
Tasmanian cattle selected	07/06/2016	6	16.1	0.4	WSW	31	15:21	10.3	98	SW	7	997.2	15.4	78	SW	19	995.4
Tasmanian cattle selected	08/06/2016	10.3	14.5	1	WSW	39	04:03	12.4	96	WSW	17	1001.9	13	93	WNW	22	1001.7
	09/06/2016	10.2	15.7	24.8	WSW	46	20:07	11.8	97	W	7	995.6	14.6	79	SW	28	998.3
	10/06/2016	7.9	13.7	1.4	WSW	67	23:54	10.8	95	WNW	15	1009.8	10.8	87	WSW	26	1010.4
	11/06/2016	5.5	10.2	8.2	WSW	78	12:18	8	74	WSW	30	1017.2	9.3	68	SW	30	1022
	12/06/2016	4.6	14.3	1.2	W	39	15:09	9.4	86	WSW	9	1032.8	12.3	90	W	22	1031.5
	13/06/2016	9.2	15.7	0.6	NW	46	12:05	12.6	80	WNW	11	1032.8	13.5	77	NW	24	1030.7
	14/06/2016	10.8	15.5	0	WNW	54	13:31	12.4	81	NW	24	1029.4	11.7	91	SW	33	1028.7
	15/06/2016	2.1	13.3	1.8	SSW	17	17:36	5.3	98	S	9	1030.8	12.1	78	S	9	1026.9
	16/06/2016	5.2	13.9	1.8	NNW	31	16:53	10.4	97	NNW	7	1018.2	11.8	85	N	17	1012.8
	17/06/2016	10.4	15.5	5.8	SW	39	16:05	13.1	96	NNW	7	1009.3	13.7	83	SW	24	1009.4
	18/06/2016	1.8	12.8	0	S	26	13:41	7.1	89	S	15	1020.5	12.5	74	SSW	15	1020.3
	19/06/2016	4.5	14.6	0.2	E	28	14:37	10.4	78	ESE	15	1021.5	13.4	74	E	19	1017.3
	20/06/2016	3.5	13.3	0	SSW	33	13:20	4.7	92	S	11	1008.5	12.6	62	SSW	20	1003.3
	21/06/2016	2.9	14.3	0	WNW	46	23:15	7.4	95	SW	7	996.9	12.1	76	NW	13	991.8
	22/06/2016	7.1	13.5	12.2	W	74	12:46	11	79	W	28	994.4	11.6	70	WSW	31	997.1
	23/06/2016	5.6	10.1	17.6	WNW	56	03:02	9.7	96	NW	9	995	6.7	91	SW	24	992
	24/06/2016	1.4	9.3	18.8	WSW	54	13:49	3.1	97	S	2	998.9	5.8	80	S	20	1004
	25/06/2016	-0.2	13	9.8	WSW	37	14:49	3.2	99	SSW	11	1019.4	12.3	79	WSW	28	1018.8
	26/06/2016	1.3	13.6	2.4	WNW	41	23:19	4.4	99		Calm	1017.3	11.4	72	NNW	19	1013.6
Groups trucked to saleyard	27/06/2016	1.9	13	8.4	W	43	13:06	4.3	98	W	4	1014.2	11.7	79	W	17	1013.8
Sale A	28/06/2016	4.3	15.3	3.6	W	52	12:48	11.7	96	WNW	15	1018	14.4	87	WSW	24	1018.3
Sale B	29/06/2016	6	16.2	2.2	WNW	46	13:49	7.5	94		Calm	1022.7	12.2	91	NW	6	1020
First "Saleyard" kill	30/06/2016	7.4	11.7	3.6	N	61	07:02	10.6	90	N	30	1005.7	11.4	88	NNW	24	1001.4
Saleyard groups rested	01/07/2016	4.5	13.2	8.4	W	43	15:20	8.9	83	WSW	17	1015.2	12.7	82	WSW	17	1015.2
	02/07/2016	8.2	13.2	4.2	WSW	50	09:49	10.9	93	W	17	1019	12.7	75	W	17	1018.8
	03/07/2016	9.6	15.6	2	WNW	56	14:14	12.1	90	W	19	1017.4	14.1	76	WNW	37	1014.8
	04/07/2016	8.4	12.1	28.8	NW	52	03:52	10	97	NNW	11	1014.1	11.5	88	ENE	11	1013
	05/07/2016	8.2	13.2	1	SSW	22	19:56	9.7	95	S	11	1016.8	12.8	81	S	11	1015.4
	06/07/2016	4.7	15	0	SSW	24	10:17	6.6	92	S	13	1020.5	13.2	76	NE	9	1020.1
	07/07/2016	1.6	12.5	0.2	E	28	23:48	3.8	99	S	6	1025.9	10.9	69	E	20	1024.8
	08/07/2016	3.8	12.2	0	E	43	12:51	9.5	78	E	20	1023.6	11.7	84	E	22	1022.1
	09/07/2016	9.2	14.1	14.2	NE	30	05:00	10.7	96	E	13	1023.8	12.6	86	ENE	19	1022.4
	10/07/2016	8	13.3	0.6	NE	56	23:17	11.7	90	ENE	22	1020.8	12.6	87	NE	30	1014.4
	11/07/2016	10.9	13.8	18.2	NNW	67	17:29	13	79	N	30	997.2	12.8	81	NNW	37	992
	12/07/2016	7.5	11.7	11	NNW	89	11:42	11.6	83	NNW	33	986.9	10.5	63	WNW	43	986.2
	13/07/2016	3.4	10.9	14.2	WSW	85	01:21	5.4	75	WSW	41	1005.6	8.2	85	W	28	1011.9
Second "Saleyard" kill	14/07/2016	5.1	12.7	5	W	80	14:03	10.8	72	W	43	1018.5	11.8	77	W	44	1018
	15/07/2016	9.8	13.9	0.4	W	52	03:16	10.9	86	W	20	1026.9	12.1	83	W	20	1026.7