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Transport duration effects on MSA eating quality

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Executive summary

This experiment found no treatment difference in eating quality, live animal, carcass, blood, urine or objective traits for four transport times ranging from 12 to 36 hours from property to the abattoir with slaughter following overnight lairage. The one exception was a small increase in blood glucose concentration in the treatment transported for 24 hours. While there were large and concerning individual animal differences in ultimate pH and meat colour these were not aligned with treatment.

The Meat Standards Australia (MSA) grading model uses multiple inputs to predict the eating quality of individual cuts of beef. In addition to these inputs MSA uses several threshold or exclusion traits aimed at minimizing animal stress and poor quality meat. Cattle presented for MSA grading cannot be mixed immediately prior to slaughter, must be slaughtered the day after dispatch and have water available up until slaughter. At slaughter the exclusions include carcasses with ribfat < 3mm, ultimate pH > 5.7 and meat colour scores >3. The minimum ribfat measurement was designed to provide buffering against extreme cooling rates in areas of the carcass with little or no fat covering. Exclusion of high ultimate pH and carcasses with dark meat colour provided some insurance against tougher meat and also dark meat at retail.

Most cattle in Australia are trucked less than 12h to slaughter, although much of the extensive northern pastoral region requires longer periods to transport cattle to slaughter. Whilst it is theoretically possible to transport cattle up to 48h before slaughter and still be eligible for MSA under the “day after dispatch” standard this would exceed the 36h maximum ‘water deprivation time’ recommended for cattle in Australia. When practical considerations (ie loading during daylight, abattoir operating times, AQIS regulations and the general industry practice of up to 12 hours in lairage at the abattoir prior to slaughter) are taken into account, the maximum transport duration would be between 24 and 36h, followed by up to 12h rest during lairage where cattle have access to water.

The experiment was designed to test the effect of four cattle transport treatments on sensory and objective measurements of meat quality. In addition blood and urine, measurements were examined as predictors of eating quality. The experiment utilised a total of 343 cattle in a replicated design with four trucking treatments. The four replicates were undertaken on four different properties in central Queensland.

The four trucking treatments differed in the hours the cattle were trucked and rested prior to delivery at the abattoir for slaughter. The control treatment was a 12 hour transport time (T12) with comparative treatments of 24 hours on the truck as a single block (T24), 24 hours on the truck arranged as 12 hours trucking followed by a 12 hour rest on hay and water in loading yards with a further 12 hours trucking (T12~T12) and finally a 36 hours transport treatment (T36). Departure times of all treatments from the properties were timed to arrive at the abattoir at approximately the same time in the evening for slaughter the following day.

Untrained consumers were used to assess tenderness, juiciness, like flavor and overall liking on striploin samples prepared using the MSA grill protocols. Sensory scores were weighted to combine into a palatability score (MQ4). Objective meat quality

measurements comprised Minolta colour dimensions (L*, a* and b*) on the raw muscle samples with peak force and cooking loss on cooked muscle samples. Blood samples at slaughter were analysed for plasma glucose, L-lactate (L-Lac), non-esterified fatty acids (NEFA) and beta-hydroxybutyrate (BHB). A urine sample was analysed for specific gravity.

The cattle in the four replicates were typical of grass fed steers being supplied to MSA from central Queensland. Across all treatments and replicates the steers had mean carcass weights of 310 kg, eye muscle areas of 78 cm^2, and ribfat of 7mm. Mean ossification and marbling scores were 170 and 290, respectively. Mean meat colour scores for all carcasses were high at 3.2. The ultimate pH ranged from 5.3 to 6.30. Relative to basal levels reported in the literature blood parameters were normal for beta-hydroxybutyrate (BHB), elevated for non-esterified fatty acids (NEFA) and L lactate and slightly elevated for blood glucose concentrations. These mean blood traits and the range within sub-groups, along with correlations between blood parameters indicated that after a 48 hour fast and a variable transport treatment, some individual animals were in negative energy balance and were starting to mobilise their body tissues. Consumer sensory scores for tenderness, juiciness, like flavour, overall satisfaction and the combined MQ4 of striploin samples placed eating quality in the mid to low 50's indicating a mid 3 star quality under the MSA grading scheme.

Transport treatment had no effect on live or carcass traits when tested using the treatment X replicate interaction as the error term. Over the four replicates ca. 10% of carcasses were excluded from the MSA grading scheme due to low ribfat (ie <3mm). or high ultimate pH (ie pHu >5.7). The greatest exclusion occurred with high meat colour scores where ca. 30% of carcasses from all treatments were excluded from MSA grading. When the thresholds for ribfat, ultimate pH and meat colour were cumulated ca. 40% of the carcasses were excluded from each treatment. The relationship between pHu and meat colour was examined to see if it varied between replicates and treatments but no trends were obvious across the different subgroups.

There were no significant treatment effects (P>0.05) on sensory scores for the different trucking treatments whether the data was analysed as the full data set (n= 343 carcasses) or only used those carcasses which had achieved the threshold criteria for ribfat, ultimate pH and meat colour scores (n=208 carcasses). Similarly there were no significant (P>0.05) treatment effects on objective peak force, cooking loss percentage of the striploin samples and Minolta colour dimensions of the raw meat.

In this experiment there was an opportunity to examine the relationship between blood and metabolic parameters and eating quality, as this has not been tested in previous experiments. The analyses tested whether blood parameters explained any additional variation in eating quality after adjustment for those terms in the MSA model. Again the predictive power was tested using two data sets being firstly the full data set and secondly a data set containing only those carcasses which had achieved the MSA thresholds for ribfat, ultimate pH and meat colour scores.

The only blood parameter related to eating quality was glucose concentration. Higher blood glucose concentration had a lower eating quality suggesting that if animals were mobilising tissue then part of this response was increased blood glucose which

ultimately reflected in poorer eating quality. For the full data set, the addition of blood glucose concentration to MSA model traits increased the R^2 from 14 to 17% for all sensory scores. When the thresholded carcasses were excluded the accuracy of the MSA model to describe sensory traits was almost double that of the full data set with an average R^2 of 25%. In the reduced data set the addition of glucose to the MSA model whilst still significant ($P<0.05$) although it had a much smaller effect only increasing the R^2 by an average of 1.3%. In other words in carcasses that achieved the thresholds for ribfat, ultimate pH and meat colour and were eligible for MSA grading, blood glucose only had a small benefit in improving sensory score prediction.

The implication of this experiment was that extending the trucking time of cattle from 12 to 36 hours had little impact on eating quality and further that a mid transport rest break provided no benefit. There were however some concerns that between replicates there was a large variation in the proportion of carcasses that were excluded from MSA grading on the basis of low ribfat, high ultimate pH and dark meat colour scores. The large variation between replicates and the high percentage of exclusions in some replicates suggest that attention needs to be given to the preparation and handling of animals in the preloading period. Results from individual replicates indicate that it was possible to prepare, load and transport animals over considerable distances and time periods without incurring problems with high ultimate pH or dark meat.

Recommendations

The first recommendation was that increasing the trucking time for cattle from 12 to 36 hours before an overnight rest in lairage and then slaughter was unlikely to impact negatively on eating quality.

The second recommendation was that the MSA data base be monitored for the percentage of exclusions due to ultimate pH and meat colour and to a lesser extent ribfat in cattle consigned to MSA which are trucked more than 12 hours. This will quantify the extent of carcasses which fail MSA in cattle trucked long distances.

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1 Introduction

Meat Standards Australia (MSA) is an innovative meat grading scheme which uses a total quality management approach to grading (Polkinghorne *et al.* 2008 a, b). The MSA system uses commercial traits from the production (breed, sex, HGP implants, milk fed veal and saleyard status), processing (carcass suspension, carcass weight, ossification and marbling scores, ribfat depth and ultimate pH) and value adding (ageing and cooking method) sectors of the beef supply chain in an empirical model to predict eating quality of individual cuts. As discussed by Polkinghorne and Thompson (2010) this approach is unique in the world in that the algorithms used in the MSA prediction model were based on consumer testing of samples from multiple cuts and cooking methods over a range of input variables (Watson *et al.* 2008a).

In addition to the grading inputs which are used in the MSA prediction model, the system uses several threshold or exclusion traits aimed at minimizing animal stress and poor quality meat (Anon 2011). Cattle presented for MSA grading cannot be mixed in the two week period prior to slaughter, must be slaughtered the day after dispatch from the property and have water available up until slaughter. At slaughter the exclusions include carcasses with ribfat < 3mm, ultimate pH > 5.7 and meat colour scores >3 (Anon 2011). The minimum ribfat measurement was designed to provide some buffering against extreme cooling rates in areas of the carcass with little or no fat covering. Exclusion of high ultimate pH carcasses provided some insurance against tougher meat and also dark meat at retail (Lawrie 1998). The exclusion of high pH meat also reduced the variation in degree of doneness for steaks cooked to the same internal temperature (Cox *et al.* 1998). Although meat colour and ultimate pH are generally correlated, the relationship is not perfect. Therefore MSA also excludes carcasses with dark meat colour scores in order to minimize meat colour variation at retail.

Currently in Australia the MSA grading system is voluntary and largely services the domestic market. From the initial launch in 2000, the number of carcasses MSA graded has grown rapidly so that in 2011/12 in excess of 2 million carcasses were graded (Anon 2012b). Griffith and Thompson (2012) have estimated that the premiums for delivery of MSA compliant carcasses were of the order of 0.15 to 0.20 $/kg carcass weight with smaller premiums being passed onto the processing and retailing sectors. As the demand for MSA eligible cattle has increased there has been interest from the beef industry (particularly the northern cattle industry) to examine mechanisms by which more carcasses can be supplied for MSA grading.

Most cattle in Australia are trucked less than 12h to slaughter (Ferguson and Warner 2008) although much of Western Queensland and the extensive northern pastoral region require longer periods to transport cattle to slaughter. Whilst it is theoretically possible to transport cattle up to 48h before slaughter and still produce carcasses that are eligible for MSA grading this would exceed the 36h maximum ‘water deprivation time’ recommended for cattle in Australia (Anon 2012a). When practical considerations (ie loading during daylight, abattoir operating times, AQIS regulations and the general industry practice of up to 12 hours in lairage at the abattoir prior to slaughter) are taken

into account, the maximum transport duration would be between 24 and 36h, followed by up to 12h rest during lairage where cattle would have access to water.

A further commercial consideration is the common industry practice of providing an intermediate rest period within long hauls which extends the total property to slaughter period. These rest periods are generally of 12 to 24 hours duration during which period the cattle are unloaded into trucking yards and provided with ad-lib hay and water. While the rest procedure is principally aimed at improved animal welfare and maintaining live and carcass weight, allied considerations include driver rest periods and fatigue management with driver hours between 10pm and 8am. Given that cattle quickly adapt to transport conditions (Ferguson and Warner 2008) the value of rest stops during trucking on animal welfare is unlikely to be useful. It is possible that the additional stress of unloading/loading together with feeding being restricted to dry hay would afford little benefit of a rest period, certainly in terms of replenishment of glycogen reserves (Gardner *et al.* 2001).

Ferguson and Warner (2008) concluded that whilst trucking cattle longer distances may impact slightly on ultimate pH it was the loading and the initial stages of transport that were the most stressful and that after this the animal tends to adapt to transport conditions. There is some information relating extended trucking times for cattle to blood and metabolic indicators (Pettiford *et al*. 2008, Warriss *et al* 1995), although there is little in relation to meat quality outcomes. Whilst longer transport times are possible within MSA specifications, this may increase the risk of poor eating quality beef. However if this risk can be quantified and managed it could conceivably be incorporated into the MSA grading model. However, there is a realization that whilst there may be a general relationship between stress, trucking time and eating quality on a lot basis this will be a relatively blunt instrument for individual animals within these groups for use as an MSA qualifying criteria, or as a prediction input. The ideal solution would be a tool that could effectively provide an individual animal measurement of stress at slaughter or during grading that could be used to identify poor quality carcasses within trucking treatments. A recent MLA industry survey (Small and Ferguson 2011) failed to find any blood (Beta-hydroxybutyrate, L-Lactate and blood pH) or urine (osmolarity and specific gravity) measures that were related to time from farm to slaughter although extensive variation was observed within groups regardless of time, distance travelled and marketing direct to abattoir or through saleyards However again no relationship with eating quality was examined.

The experiment was designed to test the effect of four cattle transport treatments on sensory and objective measurements of meat quality. In addition blood and urine measurements were examined as predictors of eating quality, both for groups and individual carcasses. The four treatments differed in terms of transport hours with one treatment including a rest interval. The same design was replicated on four properties in central Queensland. Within replicates the commencement time for the trucking treatments was staged so that the four trucking treatments arrived at the abattoir for slaughter at the same time.

2 Materials and methods

2.1 Experimental design

This experiment utilised a total of 343 cattle in a replicated design with four trucking treatments, using cattle sampled cattle from four different properties. The four treatments differed in the hours the cattle were trucked and rested prior to delivery at the abattoir for slaughter. The control treatment was a 12 hour transport time (T12) with comparative treatments of 24 hours on the truck as a single block (T24), 24 hours on the truck arranged as 12 hours trucking followed by a 12 hour rest on hay and water in loading yards with a further 12 hours trucking (T12~T12) and finally a 36 hours transport treatment (T36). Departure times of all treatments from the properties were timed to arrive at the abattoir at approximately the same time in the evening for slaughter the following day.

The experimental design and protocols were established by a subcommittee of the MSA Pathways Committee. Animal welfare approval was obtained from the Queensland DEEDI Animal Ethics Committee (Approval number SA 2012/04/383). To the extent possible consistent with scientific method the experimental procedures sought to mirror commercial practice to ensure validity in any subsequent recommendations to industry. Therefore the four replicates were conducted using different properties to ensure a variation in cattle type, management and handling facilities.

2.2 Properties and cattle

The four properties were located in central Queensland with three within 12 hours trucking time from Brisbane and the fourth located north west of Rockhampton, approximately 14 hours trucking time from Brisbane. While breed composition differed between properties they were all grassfed bullocks typical of cattle submitted from these regions for MSA grading. Replicate 1 were commercial Santa Gertrudis, replicate 2 a company crossbred involving Charolais x Santa Gertrudis, or Brangus bulls joined to Brahman x Santa Gertrudis, or Brangus cows, replicate 3 were Droughtmasters and replicate 4 a range of crosses utilising Red Poll, Wagyu and Brahman bulls over crossbred cows bred from a Brahman base. Cattle age varied from approximately 18 to 36 months and live weight from 489 to 790 kg.

2.3 The trucking procedure

For each replicate, cattle (> 100 animals) that had been run together for more than one month were mustered the day prior to the first truck loading for replicates 1, 2 and 4, or on the day of loading for replicate 3. Cattle were mustered by horse for replicates 1, 2 and 4 and motorbikes for replicate 3. Mustering was conducted by experienced stockman with cattle being walked to the yards. Following ca. one hours rest to settle in the yards, cattle were drafted, weighed, eartagged and sequentially allocated into treatment groups.

Where possible existing property tags were utilised for identification (replicates 1 and 3) and new uniquely numbered tags applied elsewhere (replicates 2 and 4). A coloured livestock spray was applied across the withers to provide visual identification to

treatment group. In replicates 1 and 2 a three way draft was conducted with treatments T36 and T12 Y12 T12 being drafted to a common pen. Four pens, one per treatment, were utilised in replicates 3 and 4. Animal numbers per treatment were determined by optimal loading density (Anon 2008) resulting in 22 head per treatment in replicates 1, 2 and 4 and 20 head per treatment in replicate 3. At the completion of the draft cattle from the different treatments were released to individual paddock areas with good access to pasture and unrestricted water supply.

The road train used in this experiment comprised two 12.2 metre trailers which arrived at each property the evening prior to the first loading. The trailers represented a widely used type in Northern Australia and were equipped with both side and rear loading doors, electrically operated ramps to load the upper deck and front and rear doors and ramps. Each deck of the trailers was divided evenly into two pens.

The same primary driver conducted the daylight transport journeys and all property loadings and unloadings. A common second driver conducted the initial night journeys with a further two drivers engaged in the final two hour movement to the abattoir. Data loggers were taped to a mid position in the top and bottom decks of the front trailer. The rear trailer was disconnected for the initial two transport cycles.

At first light on the morning following the initial draft, treatments T12~T12 and T36 for replicates 1, 2 and 4 were yarded for loading. The truck and lead trailer were then moved into position for loading utilising the rear door for replicate 1 and side doors for subsequent replicates. The T36 group were loaded on the top deck and penned at 11 per pen for replicates 1, 2 and 4 and 10 per pen for replicate 3. The T12~T12 treatment were similarly penned on the lower deck. In replicates 1 and 2 where the 44 head from these treatments had been grouped at drafting a random 22 were loaded to each deck. For replicate 3 the four treatment groups were drafted on the morning of first transport with all groups initially loaded. The truck then moved to a second set of property yards where treatments T12 and T24 were unloaded and placed in separate paddocks. The rear trailer was then disconnected and the truck departed with treatments T12~T12 and T36 as with the other replicates.

Times were recorded for mustering and the start and finish of loading each deck. The truck then departed for a notional 12 hour journey. The routes were selected by the driver to best represent a typical journey from Western Queensland. The kms of gravel and bitumen roads were recorded and all transit times and distances recorded by an on board satellite tracking system in addition to driver logbooks.

Before sunset on the first day theT24 group were yarded awaiting return of the truck. Treatment T12~T12 were then unloaded and transferred to a spacious yard with hay provided in hay racks and ad lib fresh water. For replicates 1 and 2 the individual eartags of the T12~T12 group were noted as they were unloaded to confirm the individuals in the T36 treatment, the two treatments having been randomly loaded from a common mob. T24 cattle were then loaded on to the bottom deck previously occupied by treatment T12~T12. The T36 cattle remained on the truck. The truck then departed for a second notional 12 hour journey following a similar route to the first cycle. The T12~T12 group were observed using a night vision scope at half hourly to hourly

intervals throughout the night with the number of cattle standing, lying, drinking and eating recorded together with notes of observed behaviour.

At daylight on day two of transport, the T12 group were yarded awaiting return of the truck. On return the second trailer was connected and positioned for loading. In replicate 1 this resulted in the original front trailer becoming the new rear trailer due to the yard arrangement. To allow loading via the rear ramp both groups of cattle, treatments T36 (top deck) and T24 (lower deck) were moved forward to the new front trailer. Neither were unloaded. Side loading allowed the rear trailer to be loaded directly at replicates 2 and 3. Replicate 4 had similar side loading facilities but one animal from the 36 hour treatment was removed for welfare reasons as it had been going down during the second 12 hour cycle. To facilitate this the remaining cattle were moved back one pen, with the rear pen transferred to the empty and newly attached rear trailer, the animal removed from the top deck and the remaining 43 head moved forward to their original pen locations. The T12~T12 treatment group were then loaded to the top deck of the rear trailer and the T12 group loaded on the lower rear trailer deck. Again each group was penned evenly as 11 or 10 head per pen.

Road regulations prohibit the use of class 1 (2 x 12.2 metre trailers) road trains in a general radius of 100 km around Brisbane. To comply with this the trucking company supplied a second prime mover and driver for the final stage to the abattoir. The rear trailer was disconnected and second prime mover attached to create two single trailer double decks for the final journey leg. For replicate 1 this portion was from Warwick to Brisbane, a distance of 130 km. Replicates 2 and 3 were both converted at Toowoomba resulting in a final leg of 104 km. The location of replicate 4 in a tick zone prohibited transport via the inland route to Brisbane requiring use of the coastal route from Gracemere (adjacent to Rockhampton), a route prohibited for class 1 road trains. As a consequence the double deck portion was 682 km. The progressive truck loading procedures resulted in treatment times on truck of notionally 12, 24, 12+12 and 36 hours. Table 1 shows the times taken and the kms travelled for each treatment within each replicate.

Table 1: Trucking times from the property to the abattoir and distance for the four trucking treatments within each replicate.

Replicate	Trucking treatment							
	T12[a]		T24[b]		T12~T12[c]		T36[d]	
	Hours	Km	Hours	km	Hours	km	Hours	km
Replicate1	13.4	794	25.2	1065	24.4	1387	36.5	1659
Replicate 2	12.9	843	26.3	1438	23.0	1491	36.9	2086
Replicate 3	12.7	750	23.6	1491	22.7	1312	34.1	1948
Replicate 4	17.4	1123	30.0	2066	27.5	1681	40.1	2156

[a] T12 - travelled 12 hours from the property to the abattoir,
[b]T24 - travelled 24 hours from the property to the abattoir,
[c]T12~T12 – travelled 12 hours in a truck, offloaded into yards on feed and water for 12 hours and then reloaded for 12 hours travel to the abattoir
[d]T36 - travelled 36 hours from the property to the abattoir,

On arrival at the abattoir all treatments were unloaded and held in a single dirt floored pen with access to water but no feed, as would be normal commercial practise. In addition in this experiment it was felt that common penning would help ensure a

random order of cattle from the different treatments for slaughter as it is known that kill order can impact on eating quality.

2.4 Slaughter

Each replicate was slaughtered on a different date but at a common abattoir and at the same time of day. For each of the replicates the cattle were moved from the dirt floored pens to concrete as a group at approximately 0600h the morning after arrival and were washed in the lead up to knocking with slaughter commencing at 0940. The kill rate was in the order of 100 to 120 per hour resulting in all animals being slaughtered within a one hour timeframe. This resulted in a lairage time for the different replicates of between 9 and 12 hours.

Halal slaughter procedures were used with pneumatic stunning while in a V restrainer and electrical immobilisation immediately following exit from the restrainer during shackling. Animal eartags were recorded and correlated with abattoir carcass number. At sticking a blood sample was collected utilising a pre-numbered 5 mm Vacutainer tube within one minute of slaughter. Within 45 minutes blood samples were centrifuged for 10 minutes at 3,000 rpm. The separated plasma was then drawn off by pipette and placed in two replicated numbered vials. Urine was collected from each bladder in pre-numbered 10 mm vacutainers at the evisceration table. A number of cattle had dry bladders and so no sample could be collected. Blood and urine samples were then frozen for despatch for analysis.

2.5 Carcass grading and boning

MSA graders recorded pH and temperature data at hourly intervals commencing at chiller entry and continuing for five hours in replicate 1 and from 3 to 5 hours in the remaining replicates depending on attainment of pH6. Spray chilling was used in the carcass chillers controlled by electronic sequence. MSA graders collected full MSA grading data (Anon 2011) from all carcasses early the morning following slaughter.

Bos indicus content was not assessed on individual live animals, but rather was estimated from the relationship between hump height and carcass weight which is used in the MSA model (Watson *et al* 2008a). One striploin from each carcass was collected at boning and identified by unique individual tags. A common left or right side was used for all carcasses in each replicate. Striploins were vacuum packed and chilled for 24 hours prior to collection from the abattoir.

2.6 Sample preparation

Striploins were transported by refrigerated transport at 1˚C to the meat laboratory at Armidale NSW. Each cut was removed from the vacuum bag and denuded removing all external fat and epimysium together with muscle portions other than the *m. longissimus dorsi* (LD). Five 25mm thick steaks were then prepared from a nominated position within each striploin following MSA protocols (Anon 2008) together with a portion for objective laboratory testing. Steaks were individually wrapped in freezer film prior to packing as a set of 5 per sample. Samples were then aged for 7 days from slaughter for replicates 1,

2 and 3 and 10 days for replicate 4 at which point they were frozen and stored at -20˚C until thawed for consumer testing.

2.7 Consumer sensory testing

Consumer testing was conducted using MSA grill protocols described in detail by Watson *et al* (2008b) and Anon (2008). Briefly, groups of 20 consumers, screened to include those preferring medium doneness, aged 18 to 65 and being regular beef eaters, were each served seven steaks expected to include a broad range of eating quality. The first sample served was a starting sample and was not included in subsequent analysis. The following six samples were drawn from six product groups with each product of presumed uniform quality and expected to differ from other products. To ensure range, product from this experiment was always nested within other MSA experimental product expected to provide a high (typically tenderloin) or low (typically outside flat) score. Each sensory session included a mix of all treatments from at least three replicates to minimise potential session effects. Presentation of the six test products was controlled by a 6x6 latin square ensuring that each product was presented an equal number of times in serving order from two to seven and an equal number of times before and after each other product, which effectively balanced potential order or halo effects. Each sample was tasted by 10 consumers with two served in each of 5 different presentational order positions.

Consumers rated each sample by marking four 100 mm line scales representing tenderness, juiciness, like flavour and overall satisfaction with the scales anchored by descriptions being not tender/very tender, not juicy/very juicy and dislike extremely/like extremely for both flavour and overall satisfaction scores. The raw means of each sensory trait were calculated together with clipped means calculated by removing the highest and lowest 2 scores for each trait. An MQ4 (meat quality, 4 variable) score was calculated by summing sensory scores for tenderness, like flavour and overall satisfaction weighted by 0.3 and juiciness by 0.1 to create a combined score between 0 and 100 for clipped results following procedures described by Watson (2008a).

2.8 Blood & Urine analyses

Blood samples at slaughter were centrifuged and the harvested plasma frozen (−20 ◦C) for later laboratory determination of plasma glucose, L-lactate (L-Lac), non-esterified fatty acids (NEFA) and beta-hydroxybutyrate (BHB). Laboratory analyses of plasma were carried out as a batch sample using the Olympus AU400 automated chemistry analyser (Olympus Optical Co. Ltd, Melville, NY) and commercially available reagent kits. The reagent kits for BHB (Randox Laboratories kit, Ranbut, Cat. No. RB1007, County Antrim, United Kingdom) used the enzymatic assays of McMurray *et al.* (1984).

Glucose analysis was carried out using the glucose reagent kit (Olympus Diagnostics, Tokyo, Japan, Cat. No. OSR6121) which utilises the enzymatic method of Barthelmai and Czok (1962). The plasma L-Lac was analysed with a reagent kit (Olympus Diagnostics, Tokyo, Japan, Cat. No. OSR6193) which used an enzymatic method (Barham and Trinder 1972; Trinder 1969). Plasma NEFA concentration was analysed using a NEFA-C kit (C Kit Wako Pure Chemical Ind., Osaka, Japan; modified for the

Olympus AU400 Automated Chemistry Analyser) which uses an enzymatic method based on the protocol of Itaya and Ui (1965) and Duncombe (1964).

Urine was collected from the bladder following evisceration using a vacutainer™ (Becton Dickinson, Franklin Lakes, NJ) and then frozen. Specific gravity was measured using a Pocket PAL-1 Refractometer (ATAGO Co. Ltd., Tokyo, Japan). The bladders of 38 animals did not contain any urine and so samples could not be collected.

2.9 Objective meat quality

Objective laboratory measurements included peak force, cook loss ultimate pH and Minolta color L*, a* and b* dimensions. The methodology for these measurements has been described in detail by Perry *et.al.* (2001a).

2.10 Statistical methods

Temperature at pH6 was calculated using temperature measurements recorded either side of pH6. Temperature at pH6 was calculated using linear interpolation of temperature measurements adjusted to a pH of 6.

Dressing percentage was calculated as hot carcass weight expressed as a percentage of initial live weight recorded at the commencement of the experiment.

The MSA model estimates eating quality for individual primals using an empirical regression model in association with thresholds for ribfat, ultimate pH and meat colour (Watson *et.al.* 2008a). Given these thresholds, which are used by the MSA grading scheme, it was appropriate that the data be analysed using two data sets, a) firstly the full data set of 343 carcasses and b) secondly a reduced data set of 208 which excluded those carcass that did not meet the MSA grading thresholds for ribfat, ultimate pH and meat colour scores.

The transport effects on live, carcass, blood and urine traits, sensory and objective meat quality were analysed using a mixed model (SAS, 2003) where the transport term was tested against the random term for treatment X replicate interaction. In effect this assumed that the experimental unit for the transport effect was a transport treatment within each replicate.

The effect of glucose on eating quality was investigated using a GLM model (SAS 2003). The dependent variables were sensory scores with independent variables for estimated *Bos indicus* content, carcass weight, ribfat, ossification and marbling scores and ultimate pH (as both linear and curvilinear effects). The value of glucose as a predictor of sensory scores was evaluated by adding the glucose term to this model for both the full and reduced data sets (ie data set a and b).

3 Results and discussion

3.1 Means and variance of carcass, blood parameters, sensory and objective meat quality traits

Table 2 shows the means and ranges for live weight and carcass traits for cattle used in this experiment. Using hump and carcass weight to predict *Bos indicus* content the

average *Bos indicus* content across all treatments was 21%, but ranged widely from 0 to 100%. The mean liveweight was 603 kg with a 300 kg range in liveweight across all treatments. The mean dressing percentage was 52%, but also ranged from 46 to 60%.

The mean carcass traits were typical of grass fed steers being turned off pasture from central Queensland with characteristically low ribfat relative to carcass weight and low marbling scores. Mean meat colour scores were high at 3.2 and again there was a large range from 1.7 (this is equivalent to a meat colour score of 1C on the AUSMEAT meat colour scale) to 6.0 within the population of steers in the experiment. Whilst the mean ultimate pH was acceptably low at 5.6, there a large range with a proportion of carcasses having ultimate pH in excess of 6.0. The temperature at pH6 (or rigor temperature) showed that whilst the average rate of pH decline was considered optimal (Thompson *et al.* 2006), there was a wide variation between individual carcasses with a range from 13 to 35^{o}C.

Table 2 Raw means, variance and ranges for live weight, dressing percentage and carcass measurements for steers in the four trucking treatments

Trait	Mean	Stdev	Min	Max
Est *Bos indicus* content	21	22	0	100
Liveweight (kg)	603	50	489	790
Dressing %	51.5	2.2	45.5	59.5
Carcass weight (kg)	310.6	25.1	243	391
Ribfat (mm)	6.6	2.8	1.0	19.0
Eye Muscle area (cm^2)	77.6	7.7	58.0	100.0
Ossification score	169	33	110	350
MSA Marbling score	291	73	150	530
Meat Colour score	3.2	0.9	1.7	6.0
Ultimate pH	5.55	0.12	5.32	6.30
Temp@pH6 (^{o}C)	24.5	5.1	13.1	35.3

There are a number of blood metabolites that can be used to assess the metabolic status of the animal. Beta-hydroxybutyrate (BHB) and non-esterified fatty acids (NEFA) are commonly used as indicators of negative energy balance in the body leading to tissue mobilisation. Elevated BHB and NEFA concentrations indicate that the supply of glucose was limiting and animals were starting to rely on fat mobilization to maintain glucose for metabolism. BHB is specifically an indicator of elevated ketone bodies, whilst NEFA are a measure of fat breakdown products. Glucose concentration is an indicator of the circulating glucose to support metabolism of tissues in the body. If the body is under nutritional stress the breakdown of glucose to pyruvate and lactate exceeds the ability of the animal to clear these metabolities from the blood and L lactate levels will appear elevated. Specific gravity of the urine is an indicator of the dehydration status of the animal.

BHB concentration appeared to be normal with a mean concentration of 0.19 mM/L (Table 3). These concentrations were similar to the levels recorded by Stockman *et al.* (2011) in naïve and habituated cattle prior to imposition of transport treatments. The

range of BHB would indicate that some animals were starting to mobilise their tissues and produce ketone bodies. In contrast mean NEFA levels were 0.43 mM/L which was a 10 fold increase relative to the basal level reported by McGilchrist *et al.* (2011). Both the mean glucose and lactate levels appeared to be elevated compared to animals that had not undergone fasting and transport stress. In this study mean levels of 6.9 mM for glucose and 9.4 mM for L lactate were recorded compared to the basal levels of 4.5 and 0.4 for glucose and L lactate reported by McGilchrist *et al.* (2011). The base levels for glucose reported by Stockman et al (2011) at 5.5 mM/L were higher than McGilchrist *et al.* (2011) but still lower than the mean concentration of 6.9 mM/L reported in this experiment (Table 3). Again the upper ranges for glucose and L lactate would indicate that after nearly 48 hours fasting there were a number of individual animals that were mobilising their tissues. Perhaps counterintuitively there was no association of these individuals to transport treatment.

Specific gravity of the urine appeared normal. The number of dry bladders (n=38) was not considered abnormal as this could simply indicate that the animals had urinated immediately prior to slaughter.

Table 3 Raw means, variance and ranges for the concentration of Beta-hydroxybutyrate, glucose, non-esterified fatty acids, L lactate in the blood and the specific gravity of urine in cattle used in the experiment

Trait	Mean	Stdev	Min	Max
Beta-hydroxybutyrate (mM/L)	0.19	0.07	0.05	0.44
NEFA (mM/L)	0.430	0.194	0.086	1.208
Glucose (mM/L)	6.85	1.10	4.71	11.61
L-Lactate (mM/L)	9.44	3.24	3.93	23.22
Urine SG	1.009	0.009	1.000	1.040

Table 4 showed the correlation coefficients between blood and urine metabolites. As expected BHB and NEFA concentrations were positively correlated ($r=0.77$, $P<0.05$). Those animals that were metabolising tissues as indicated by elevated BHB concentrations tended to also have lower glucose and L lactate concentrations. However breakdown of fat as indicated by elevated NEFA was not correlated with glucose but was negatively correlated to L lactate concentrations. As expected glucose and L lactate concentrations were positively correlated indicating that animals that had higher glucose concentrations in the blood to support metabolism of tissues also had difficulty in clearing the lactate from the blood and so it was also elevated ($P=0.049$, $P<0.05$).

Table 4 Correlation coefficients for blood (Beta-hydroxybutyrate BHB, non-esterified fatty acids NEFA, glucose and L lactate) and urine (specific gravity) parameters

	BHB	NEFA	Glucose	L Lactate	Specific gravity
BHB	1.00	**0.77**	**-0.18**	**-0.38**	0.07
NEFA		1.00	-0.05	**-0.30**	0.05
Glucose			1.00	**0.49**	-0.06
L lactate				1.00	-0.06
Urine Specific gravity					1.00

Bolded correlations indicate significance at P<0.05

Mean sensory scores for the grilled striploin samples are shown in Table 5. Generally all sensory scores were in the mid to low 50's indicating the striploins would have graded in the lower proportion of the MSA three star grade. Juiciness had the highest mean sensory score with mean tenderness scores being the lowest. The range in all quality dimensions stood out with scores varying from 20 to mid to high 80s. This variability of sensory scores within the experiment was reflected in the high coefficients of variation of 20 to 30% for the sensory scores.

Table 5 Means and ranges for sensory scores (tenderness, juiciness, like flavor, overall liking and MQ4 scores) of the grilled striploin samples along with objective measurements of meat quality which included peak force, cooking loss and Minolta colour (L*, a*, b*)

Sensory Traits	Mean	Stdev	Min	Max
Sensory scores				
Tenderness	51.9	14.4	16	84
Juiciness	56.2	12.3	22	85
Like Flavour	54.4	11.3	21	84
Overall Liking	54.1	12.6	18	87
MQ4	53.4	12.0	21	83
Objective Meat Quality				
LD Shear Force (kg)	5.1	1.4	2.2	9.6
% cooking Loss	24.4	3.4	15.4	32.2
Minolta L*	35.1	2.9	27.2	44.2
Minolta a*	19.7	2.1	12.0	24.8
Minolta b*	9.0	1.4	4.6	12.7

Means and variance for the peak force of the striploin muscles is shown in Table 5. Again a mean shear force of over 5 indicated the striploins were average to low quality which aligned with the sensory scores. As expected the shear force also had a coefficient of variation of 27% indicating a high variability in the measurement. Whilst shear force was negatively related to sensory scores, the strength of the relationship was only low (R^2 of 10.6%), which was considerably less than the 60% reported by

Perry *et al.* (2001b) In contrast the variation in cooking loss and colour dimensions was much less with coefficients of variation 8 to 15%.

3.2 Transport effects on carcass traits, blood parameters, sensory and objective meat quality measurements

Table 6 shows the predicted means for the live animal and carcass traits when tested in a model where the trucking effects were tested against the treatment X replicate interaction. For all of the live animal and carcass traits in Table 6 the trucking treatments failed to achieve significance (P>0.05). The lack of a treatment effect and the care taken to randomize animals to treatment at the commencement of the experiment was reflected in the similarity between the treatment means.

Table 6 Predicted means for live animal and carcass traits for the four transport treatments when tested against the treatment X replicate interaction

	Transport treatments				Av	Sign
Trait	T12	T12~T12	T24	T36	SE	
Est *Bos indicus* %	23.8	18.8	21.7	23.3	10	NS
Liveweight (kg)	599	603	607	606	19	NS
Dressing %	51.7	51.3	51.7	51.3	0.8	NS
Carcass weight (kg)	309	309	314	310	9	NS
Ribfat (mm)	6.6	6.9	6.7	6.1	0.4	NS
Eye Muscle area (cm^2)	77.8	76.2	78.5	77.9	0.8	NS
Ossification score	169	171	167	171	10	NS
US Marbling score	287	285	300	290	19	NS
Meat Colour score	3.2	3.3	3.2	3.2	0.3	NS
Ultimate pH	5.56	5.57	5.56	5.55	0.03	NS
Temp@pH6 (^oC)	23.2	25.2	24.6	24.9	0.7	NS

See Table 1 for treatment abbreviations

NS –the treatment effect was not significant (P>0.05) when tested against the treat X replicate interaction

Table 7 shows the percentages of carcasses excluded from the MSA grading scheme for the 16 treatment x replicate subgroups based on the thresholds for low ribfat,, high ultimate pH and meat colour. The exclusions were presented for each of the treatments within each replicate and then when these numbers were cumulated for each of the threshold traits. When the percentages of exclusions were averaged across the four replicates it was possible to test the significance of the treatment against the treatment X replicate interaction.

Between replicates the percentage of exclusions due to low ribfat (ie < 3mm) varied from 0 to 23% (Table 7). Replicates 1 and 2 appeared to have higher percentages of exclusion due to low ribfat (ca. 16%) compared with replicates 1 and 2 where the average percentage of exclusion across the four treatments was of the order of only 4%. When averaged over the four replicates exclusion due to low ribfat was of the order of 10% and did not differ significantly between trucking treatments (Table 7). Given that cattle were exposed to a relatively short period of trucking stress (anywhere from 12 to

36 hours of trucking) it was unlikely that transport would have had any effect on ribfat. Rather it was likely that the high levels of exclusions between replicates due to low ribfat reflected poor selection of animals to be consigned to slaughter. On a national basis exclusion from MSA grading due to low ribfat was of the order of 1% (Anon 2012b), indicating that low levels of exclusion due to low ribfat are achievable.

A similar pattern of large variation between replicates in the percentage of carcasses excluded on high ultimate pH was evident in Table 7. For this trait the percentage of exclusions was higher in replicates 1 and 3 (ca. 16%) compared to replicates 2 and 4 (ca. 4% exclusions). Within replicates there were often large differences between treatment sub-groups (see replicate 3, Table 7) but when viewed across the four replicates there was no obvious pattern and when the average treatment effect was tested on the treatment X replicate error term the different trucking treatment effects were not significant (P>0.05).

Table 7 Percentages of carcasses which failed MSA based on threshold levels for ribfat (<3mm), ultimate pH >5.7) and meat colour scores (>3 meat colour) as individual traits and collectively for each replicate x treatment subgroup and then meaned for the four trucking treatments.

Exclusion		Transport Treatment					
Trait	Rep	T12	T12~T12	T24	T36	Av SE	Sign
Ribfat <3mm	1	22.7	9.1	9.5	22.7		
	2	9.1	9.1	22.7	22.7		
	3	5.0	0.0	0.0	10.0		
	4	4.5	9.1	4.5	0.0		
	Mean	**10.3**	**6.8**	**9.2**	**13.9**	**4.4**	**NS**
pHu >5.7	1	18.2	13.6	9.5	18.2		
	2	0.0	4.5	4.5	0.0		
	3	30.0	5.0	25.0	0.0		
	4	0.0	4.5	9.1	4.8		
	Mean	**12.0**	**6.9**	**12.0**	**5.7**	**4.9**	**NS**
Meat Colour	1	27.3	27.3	4.8	27.3		
	2	4.5	13.6	4.5	0.0		
	3	90.0	70.0	80.0	75.0		
	4	22.7	13.6	27.3	23.8		
	Mean	**36.1**	**31.1**	**29.2**	**31.5**	**16.5**	**NS**
All 3 exclusion	1	40.9	36.4	23.8	36.4		
Traits	2	13.6	22.7	27.3	22.7		
	3	90.0	70.0	80.0	75.0		
	4	22.7	22.7	31.8	23.8		
	Mean	**41.8**	**38.0**	**40.8**	**39.5**	**13.6**	**NS**

See Table 1 for treatment abbreviations,

NS –the treatment effect was not significant (P>0.05) when tested against the treat X replicate interaction

Within treatment/replicate subgroups the percentages of carcasses excluded due to high meat colour scores was generally much higher, but again there was large differences between replicates. Replicate 2 had a relatively low level of dark meat colour with only 6% of the carcasses excluded, compared with 22% in replicates 1 and 4. Replicate 3 had the highest proportion of exclusions across the four treatments with almost 80% of carcasses excluded on the basis of dark meat colour scores (see Table 7). Again there was no obvious pattern between treatments in the exclusions due to high meat colour. Therefore as expected when the treatment effect was tested against the treatment x replicate error term, the different trucking treatments had no significant effect on the percentage of exclusions due to dark meat colour scores ($P>0.05$).

The percentages of dark meat colour scores in replicate 3 ranged from 70 to 90%. This extremely high level of dark meat colour at grading raised concerns that perhaps the carcasses had not reached ultimate pH and this restricted the formation of oxymyoglobin on the exposed muscle surface. To address this concern striploins were rescored at cut up two days later which confirmed the extremely high levels of dark meat colour scores in this replicate (R Polkinghorne, *unpublished data*).

When all three thresholds (ie ribfat, ultimate pH and meat color scores) were applied the percentage of carcasses that were excluded for MSA grading was of the order of 40%. As expected there was a positive correlation between ultimate pH and meat colour ($r=0.62$, $P<0.05$), but no relationship between ultimate pH and ribfat ($r=-0.09$, $P>0.05$). Ribfat and meat colour were negatively correlated ($r=-0.16$, $P<0.05$), possibly because higher ribfat was related to higher marbling which effectively would tend to decrease meat colour scores. Again the percentage of carcasses excluded due to a combination of low ribfat, high ultimate pH and dark meat colour varied enormously between replicates with replicates 2 and 4 having ca. 23% of carcasses excluded followed by replicate 1 with 34% and replicate 3 having the highest percentage of exclusions at 79%.

The levels of exclusions in this study for ultimate pH and particularly meat colour were much higher than the national average reported by MSA (Anon 2012b). For ultimate pH and meat colour scores the national average of exclusion from the MSA grading scheme was of the order of 2.5 to 4.5% (Anon 2012b), compared with percentage exclusions as high as 30 and 90% for high ultimate pH and meat colour respectively.

Although ultimate pH and meat colour were correlated the relationship was not perfect and replicate 3 had much higher levels of dark cutting than would be expected from the ultimate pH readings. This suggested there may have been a breakdown in the normal relationship between meat colour and ultimate pH. However when the relationship between meat colour scores and ultimate pH was examined within the 16 subgroups, it was found to be significant and positive in almost all subgroups and replicate 3 did not stand out as having a different relationship between meat colour and ultimate pH. Whilst it was difficult to ascribe possible reasons for the higher meat colour scores in this replicate the high levels would present a real economic cost to producers delivering to MSA.

The two replicates with greater ultimate pH and meat colour failures generally aligned with behavioural observation of the cattle being less docile in replicates 1 and 2 than

replicates 2 and 4, but in each case there is no evidence of a difference between treatments within the replicates. Replicate 3 cattle were mustered, drafted and loaded on the same day whereas the other replicates had all groups rested on pasture overnight between drafting and loading, possibly contributing to the excessive meat colour scores in this replicate. The T12 and T24 groups in replicate 3 were also trucked between property yards resulting in an additional unloading and loading but, given the lack of difference with the T36 and T12~T12 groups, this appears to have had no additional impact.

General overnight observation of the T12~T12 groups for all replicates also raised some questions in regard to what might be expected during a rest break. The number of cattle that lay down to rest, ate hay or drunk varied considerably with some, replicate 2 in particular, consuming virtually all hay on offer whereas the other extreme was replicate 1 which consumed essentially no hay with a maximum of 2 head observed eating at any half hour observation. Few cattle were observed drinking in any replicate. The resting behavior also varied with generally less than one third of the group observed lying down at any one time within any replicate, with replicate 1 being the exception with ca. 2/3 observed lying down through the night. Standing behavior ranged from apparent resting in a relatively tight group to near continuous restless movement across the replicates.

Table 8 showed the predicted means for the blood parameters and urine specific gravity when tested in a model which tested the significance of the treatment effects against the treatment X replicate interactions. For BHB, NEFA and L-lactate concentrations and urine specific gravity there was no significant effect of trucking treatments (P>0.05). However treatment effect was significant (P<0.05) for glucose concentration, whereby treatments T12~T12 and T36 had lower blood glucose levels than the T24 treatment. The higher glucose concentrations in T24 are difficult to explain as animals in the T36 treatment were exposed to a longer period of transport. Whilst an elevated glucose concentration may indicate a response to stress it should be noted that the trucking differences in glucose concentration were rather small and only just achieved significance.

Table 8 Predicted means for blood Beta-hydroxybutyrate (BHB), non-esterified fatty acids (NEFA), glucose, L lactate concentrations and urine specific gravity (SG) for the four transport treatments when tested against the treatment X replicate interaction

	Transport treatments				Av	Sign
Trait	T12	T12~T12	T24	T36	SE	
BHB (mM)	0.17	0.21	0.18	0.21	0.01	NS
NEFA (mM/L)	0.37	0.43	0.43	0.49	0.04	NS
Glucose (mM/L)	6.86^{ab}	6.68^{a}	7.23^{b}	6.60^{a}	0.14	*
L-Lactate (mM/L)	9.29	9.92	9.79	8.74	0.39	NS
Urine SG	1.009	1.011	1.008	1.010	0.001	NS

NS - not significant at P>0.05, * - significant at P<0.05 when tested against the treat X rep interaction
See Table 1 for treatment abbreviations,

As discussed previously the MSA model uses several exclusion factors to determine whether a carcass is eligible for grading. With this in mind the analysis of the effect of

trucking treatments on sensory scores was undertaken in two stages. The first comprised data from all animals (n=343 carcasses) and the second only data from those carcasses which passed the MSA exclusion thresholds (n=208 carcasses).

Table 9 showed the predicted means for sensory scores using the full and reduced data sets. Whether tested using the full or reduced data sets trucking treatments were not significant for any of the sensory scores when tested against the treatment X replicate interaction term (P>0.05). It was of interest that the means for the different trucking treatment were similar regardless of whether the data were analysed as the full data set, or as the subset which excluded those carcasses that were excluded because of threshold traits in the MSA model. In other words the threshold factors of low ribfat, high ultimate pH and dark meat colour did not target low eating quality samples, but rather those samples where the cuts from the carcasses would have been unacceptable to the consumer. As discussed by Viljoen et al. (2002) whilst dark cutting meat is clearly discriminated in the fresh form when consumers are selecting beef, little difference was found in palatability characteristics between normal and high pH/dark meat when cooked and served to untrained consumers.

Table 9 Predicted means for striploin sensory scores (tenderness, juiciness, like flavor, overall liking and MQ4 scores) for the four transport treatments. The treatment effect was tested on the treatment X replicate interaction. Analyses were undertaken using a) all data which comprised 343 and b) a subset of 208 carcasses which excluded those that exceeded MSA thresholds for low ribfat, high ultimate pH and dark meat colour scores.

Sensory	Data	Transport treatments				Aver	
Traits	Set	T12[a]	T12~T12	T24	T36	Se	Sign
Tenderness	a) all data	54.1	51.7	49.0	52.7	3.2	NS
	b) subset	52.8	50.1	50.6	54.3	3.6	NS
Juiciness	a) all data	58.1	56.4	53.6	57.0	3.2	NS
	b) subset	56.0	55.3	55.0	57.7	3.8	NS
Like Flavour	a) all data	55.6	53.9	53.2	55.0	2.2	NS
	b) subset	54.4	52.1	55.4	56.1	2.8	NS
Overall Liking	a) all data	56.2	53.8	52.0	55.2	2.6	NS
	b) subset	54.2	51.8	54.1	56.9	3.2	NS
MQ4	a) all data	54.9	53.2	51.3	54.3	2.6	NS
	b) subset	53.6	51.6	53.1	55.5	3.2	NS

[a] See Table 1 for treatment abbreviations,

NS –the treatment effect was not significant (P>0.05) when tested against the treat X replicate interaction

Table 10 showed the predicted means for peak force and cooking loss percentage along with Minolta colour dimensions L*, a* and b* using the full and reduced data sets. In all cases whether tested using the full data set or reduced data set the trucking treatments were not significant (P>0.05) for any of the objective measurements and

colour dimensions when tested against the treatment X replicate interaction term (P>0.05). It was interesting that when the high pH and high meat colour carcasses were excluded from the data set there was very little effect on Minolta colour dimensions. Although not reported here the difference in Minolta colour dimensions for included and excluded carcasses was examined and it was found that those excluded carcasses had significantly (P<0.05) lower L*, a* and b* dimensions. In other words the carcasses that were excluded because of low ribfat, high ultimate pH and dark meat colour scores were darker (lower L*), less red (lower a*) and less yellow (lower (b*) than the carcasses that achieved these thresholds.

Table 10 Predicted means for striploin peak force (LDPF), cooking loss percentage and Minolta meat colour dimensions (L*, a*, b*) of the raw meat for the four transport treatments. The treatment effect was tested on the treatment X replicate interaction. Analyses were undertaken using a) all data which comprised 343 and b) a subset of 208 carcasses which excluded those that exceeded MSA thresholds for low ribfat, high ultimate pH and dark meat colour scores.

Sensory	Data	Transport treatments				Aver	
Traits	Set	T12[a]	T12~T12	T24	T36	Se	Sign
LDPF (kg)	a) all data	4.9	4.9	5.3	4.9	0.4	NS
	b) subset	4.9	4.9	5.2	4.9	0.4	NS
Cooking loss%	a) all data	24.2	24.2	24.4	24.1	1.4	NS
	b) subset	24.4	24.5	24.0	24.5	1.5	NS
Minolta L*	a) all data	35.1	35.3	35.2	35.4	1.2	NS
	b) subset	35.3	35.7	35.5	36.0	1.2	NS
Minolta a*	a) all data	19.4	20.0	19.9	19.6	0.4	NS
	b) subset	19.9	20.4	20.4	19.8	0.5	NS
Minolta b*	a) all data	8.9	9.1	9.0	8.8	0.2	NS
	b) subset	9.3	9.3	9.4	8.9	0.3	NS

See Table 1 for treatment abbreviations, NS –the treatment effect was not significant (P>0.05) when tested against the treat X replicate interaction

3.3 Other predictors of eating quality

Whilst there are several studies which examine the effect of fasting stress and trucking times on blood and metabolic indicators (Pettiford *et al*. 2008, Warriss *et al* 1995), there is little information in relation to whether these parameters have any relationship to eating quality. In Table 3 BHB concentrations appeared to be consistent with basal levels reported in the literature. In this experiment NEFAs and L-lactate concentrations were elevated relative to basal levels in the literature, with only a slight trend for elevated blood glucose concentrations. Also in Table 4 the correlations between blood parameters indicated that after 48 hours fasting and after being subjected to different

transport treatments there were individual animals within all treatments that were obviously undergoing some degree of tissue mobilisation.

The question of whether blood parameters were related to eating quality was addressed in this study using an analysis which adjusted for the input terms that are included in the MSA model and then adding blood parameters to test whether they accounted for any more of the variance in sensory scores. The F ratios for the terms in the model using a) the full data set of 343 carcasses and b) the reduced group of 208 carcasses after excluding those with low ribfat, high ultimate pH and high meat colour scores are shown in Table 11. In addition Table 11 shows the coefficient of determination and residual standard deviation for prediction of sensory scores using these different models.

After adjustment for input variables in the MSA model blood glucose concentration was the only blood parameter that accounted for variation in sensory scores. Blood BHB, NEFA, L-Lactate and urine specific gravity were not significant for any of the sensory scores.

There are several important points that need to be taken from Table 11. Firstly whilst the glucose concentration term had higher F ratios when using the full data set compared with the reduced data set, the prediction of sensory scores was much less accurate when using the full data set compared with the reduced data set. This was due to the carcasses that were excluded from grading because they exceeded the MSA threshold values were more variable in their sensory scores than those that complied with the grading criteria. When all the data was used the addition of glucose concentration to the MSA prediction model increased the coefficient of determination of prediction equations from 13.7 to 16.8 (and conversely decreased the residual standard deviation) showing an average increase in accuracy of 3.1%. For the reduced data set the accuracy of using MSA traits was much higher (and the residual standard deviation lower) and the impact of adding the glucose concentration term to the MSA prediction model, whilst still significant, had a much smaller F ratio.

The second point to be taken from Table 11 was that excluding the carcasses that failed to achieve the thresholds for ribfat, high ultimate pH and high meat colour effectively removed much variability from the sensory scores. The prediction of the MSA model was increased almost two fold with a corresponding decrease in the residual standard deviation. Despite this reduction however there remains unexplained individual animal variance not detected by MSA grading inputs or the exclusions.

Warner *et.al* (2007), in an experiment testing the impact of stress imposed via electric prodders immediately prior to slaughter, reported an increase in plasma lactate and decrease in MQ4 score and other sensory traits together with reduced water holding capacity without any change in meat colour or pH decline and ultimate pH. This continues to indicate the need for a further definitive test that may indicate individual animal stress at slaughter and able to be applied as a cut-off or additional grading input.

The other approach taken to understand the potential role for blood glucose concentrations in the MSA grading scheme was to investigate the use of glucose concentration as a threshold factor in carcasses that were at risk of poor eating quality after a long distance road trip. To examine this approach carcasses were sorted into those that were acceptable for MSA Grading (ie had satisfied all the threshold criteria)

and those carcasses that had been excluded from MSA Grading because they had failed the threshold criteria on low ribfat, high ultimate pH and high meat colour.

The graphs in Figure 1 show the relationships between MQ4 score and blood glucose concentration. In the two data sets which comprised carcasses a) included and b) excluded from grading it can be seen that if a threshold line was drawn at 10 or 8 mM/L glucose concentration some low eating quality carcasses (MQ4 < 30) could be identified but there were many other carcasses with MQ4 scores of ca. 20 which were not identified by this threshold. In fact to exclude these carcasses from either data set a glucose concentration threshold of less than 6 would be required which would eliminate a large proportion of good eating quality carcasses.

Therefore it was concluded that in the presence of other MSA inputs, whilst blood glucose concentration did improve the accuracy of predicting sensory score the increase in accuracy was marginal, particularly in those carcasses which achieved the threshold criteria of low ribfat, high ultimate pH and high meat colour scores. In those carcasses excluded from MSA because they had not passed the threshold, blood glucose concentration was perhaps more useful, although it should be remembered that the vast majority of these carcasses were excluded because they were unacceptable in terms of meat colour. Given the consumers discrimination against dark meat colour, identification of poor eating quality carcasses in this subgroup may be of little practical value.

Table 11 F ratios, coefficients of determination (R^2)and residual standard deviations (RSD) for the effect of MSA input traits (estimated *Bos indicus* content, HGP, carcass weight, ribfat, ossification and marbling scores and ultimate pH) and blood glucose concentration on sensory scores using a) the full data set of 343 carcasses and b) the reduced data of 208 carcasses which removed those animals excluded from being graded due to low ribfat, high ultimate pH and high muscle colour scores.

	Sensory scores									
	Tend[a]	Tend	Juicy	Juicy	Flav	Flav	Overall	Overall	MQ4	MQ4
	Traits	Traits + gluc	Traits	Traits + gluc	Traits	Traits + gluc	Traits	Traits + gluc	Traits	Traits + gluc
a) All data (n=343)										
Est BI	**4.8**	3.3	**4.1**	2.6	2.9	1.8	**5.4**	**3.7**	**5.1**	3.50
HGP	**9.4**	**6.9**	**21.9**	**18.7**	**7.2**	**5.4**	**10.3**	**7.8**	**10.7**	**8.2**
Car wt	**12.9**	**10.6**	**8.2**	**6.1**	**4.9**	3.5	**8.7**	**6.4**	**9.8**	**7.5**
Ribfat	2.8	2.7	**6.4**	**6.2**	2.5	2.3	**4.3**	**4.1**	**4.1**	**3.9**
Uoss	1.0	1.3	0.2	0.4	3.0	3.6	2.4	3.0	1.8	2.4
UMb	**25.4**	**26.5**	**27.9**	**28.5**	**36.2**	**37.0**	**36.2**	**37.5**	**35.1**	**36.2**
pHu	**13.0**	**10.7**	**9.5**	**8.6**	**4.6**	**3.9**	**7.4**	**7.3**	**8.9**	**7.54**
pHu^2	**12.7**	**10.5**	**9.2**	**8.4**	**4.4**	2.8	**7.2**	**6.1**	**8.6**	**7.39**
Glucose		**12.4**		**10.9**		**12.7**		**15.6**		**14.0**
R^2	13.7	16.6	12.4	14.9	13.3	16.4	14.5	18.2	14.5	17.7
RSD	13.6	13.4	11.6	11.5	10.7	10.6	11.8	11.5	11.2	11.1
b) Subset of data (n=208)										
Est BI	**7.5**	**7.0**	**4.0**	3.2	**7.5**	**6.7**	**9.4**	**8.4**	**8.9**	**8.1**
HGP	**4.3**	2.8	**14.6**	**13.0**	**4.3**	**3.5**	**4.7**	**3.4**	**5.8**	**4.4**
Car wt	**3.7**	3.4	2.4	2.2	1.3	1.2	2.0	1.8	2.8	2.6
Ribfat	**4.2**	**4.7**	**6.4**	**6.4**	**6.6**	**6.8**	**7.3**	**7.7**	**6.3**	**6.6**
Uoss	**3.6**	**4.0**	1.4	1.7	**8.3**	**9.0**	**5.4**	**6.1**	**5.4**	**6.0**
UMb	**23.9**	**24.1**	**24.3**	**23.8**	**32.5**	**32.3**	**30.0**	**30.0**	**30.1**	**30.1**
pHu	**5.1**	**5.0**	**14.0**	**13.5**	3.7	**4.2**	**4.9**	**4.9**	**6.1**	**6.2**
pHu^2	**5.0**	**4.9**	**13.8**	**13.2**	3.6	**4.2**	**4.8**	**4.8**	**6.0**	**6.1**
Glucose		**4.3**		3.0		**4.0**		**4.5**		**4.1**
R^2	24.3	25.8	24.2	24.7	25.5	27.0	25.6	27.1	26.3	27.7
RSD	12.5	12.4	11.1	11.1	9.9	9.9	11.0	10.9	10.4	10.4

[a] The five sensory scores were tested using models without and with glucose. For a) the full daa set the terms in these models were tested on 1,334 df for the model with MSA traits and 1,329 df for the model with MSA traits and glucose and for b) the reduced data set terms were tested on 1,199 df for the model with MSA traits and 1,196 df, for the model with MSA traits and glucose respectively. Those F ratios that were bolded were significant at P<0.05

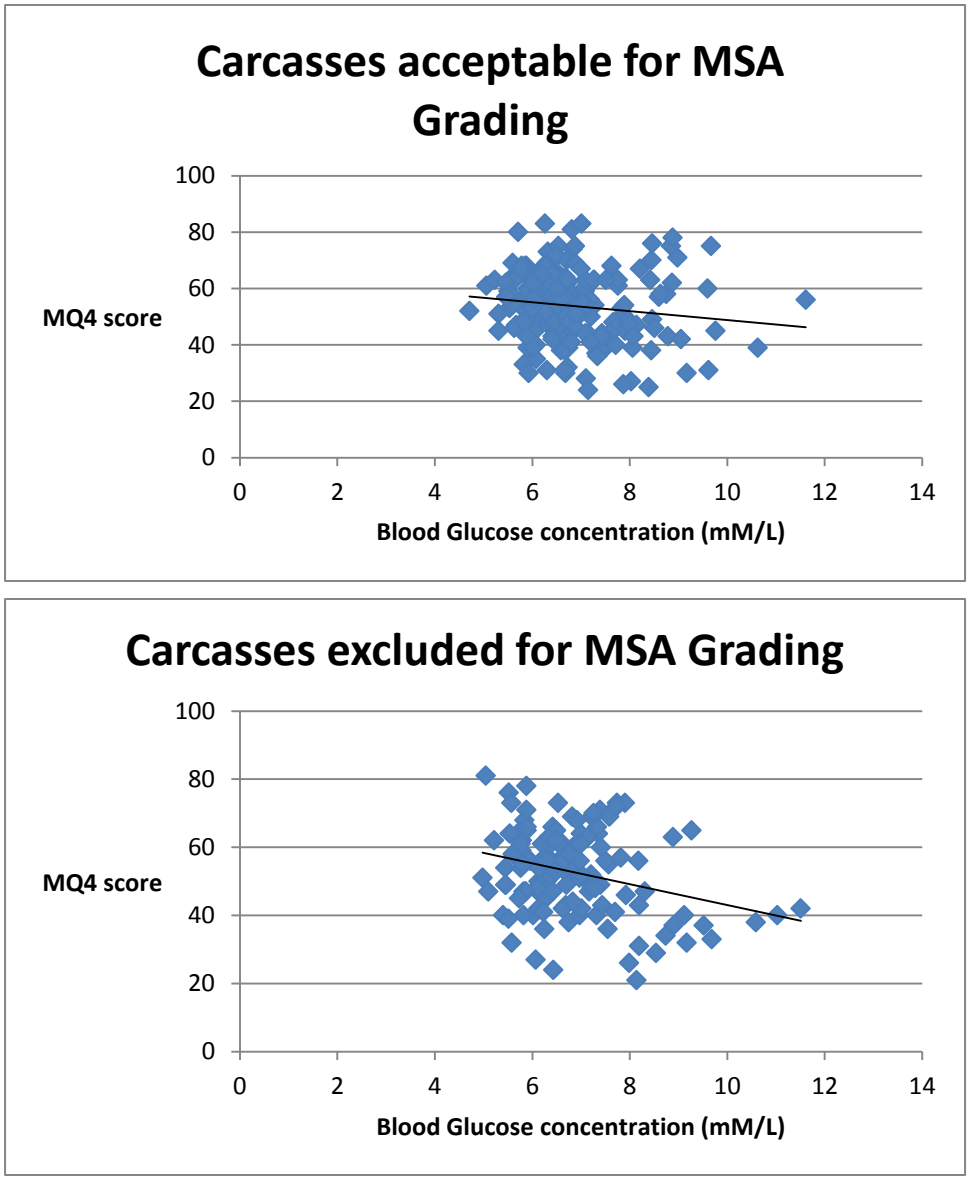

Figure 1 Graphs of MQ4 scores against blood glucose for a) those carcass that had achieved the threshold criteria and were acceptable for MSA Grading and b) those carcass that had failed the threshold criteria and were not acceptable for MSA Grading

4 Implications

This experiment monitored eating quality on groups of cattle subjected to a range of trucking treatments prior to slaughter. In particular the experiment examined whether extending trucking time from 12 to 36 hours had a detrimental effect on eating quality, and also whether offloading the cattle part way through the transport period for a 12h rest break on water and hay had a beneficial effect.

The results clearly indicated that extending the transport time from 12 to 36 hours did not have a detrimental effect on eating quality. Similarly the provision of a break in an extended road trip where cattle were rested on water and hay had no perceivable benefit in terms of eating quality or dressing percentage. This is contrary to the opinion of a number of experienced commercial cattlemen and perhaps warrants further validation through monitoring additional typical consignments with a rest versus continuous trucking control. Often in industry the rest period extend to 24 hours, compared to the 12 hours used in this study and this extended period could be examined in future work.

The high proportion of carcasses that were excluded from being graded, particularly because of high meat colour, was of great concern. The average percentage of carcasses that failed MSA because of exclusions of ribfat, high pH and meat colour was ca. 40% which in a commercial environment would be a serious consequence. The rate of exclusions did not differ between treatments but there was a large variation between the replicates in the proportions of carcasses excluded for the different thresholds.

A ribfat threshold failure may be addressed by greater attention to drafting the cattle on ribfat thickness and only consigning lots where all cattle exceed the minimum fat thickness of 3mm. In replicates 1 and 2, 16% of the cattle were excluded because of low ribfat compared to the national average of ca. 1%. This may reflect seasonal pressure and the desire to accommodate the experiment at a less than ideal time in relation to available cattle. In replicates 3 and 4 the proportion of carcasses which were excluded on ribfat was of the order of 5% which aligns more closely with industry averages.

The variation in ultimate pH between replicates is a greater concern. Replicates 1 and 3 had ca. 16% of the carcasses excluded because of high ultimate pH compared with only ca. 4% in replicates 2 and 4, respectively. Whilst replicates 1 and 3 had very high percentages of unacceptable ultimate pH, those for replicates 2 and 4 indicate that, even with long distances and transport times, a low failure rate is achievable if cattle have sufficient muscle glycogen at the start of the transport treatment and are not stressed during transport. It was noted that the cattle in replicates 1 and 3 were more flighty during handling which may have contributed to a pre-loading depletion of glycogen and resultant higher ultimate pH. While the double trucking of treatments T12 and T24 in the replicate 3 was of concern it appears to have had no impact as results for the standard T12~T12 and T36 treatments remain constant in common with other subgroups within that replicate.

The high variation in meat colour scores between replicates is the most concerning result of this experiment. Replicate 2 had the lowest percentage of carcasses excluded on meat colour at 6%. Replicates 1 and 4 were the next highest at 22%, followed by replicate 3 at ca. 80% of carcasses excluded on meat colour. These percentages, particularly for replicate 3, were far too high to be commercially sustainable.

The other point to be made is that the ambient temperatures were considered ideal for transporting cattle with no rain being recorded during the trucking periods for any of the replicates. Also the drivers were all experienced and the truck logs indicated that the

driving was not abrupt or placed undue stress on the animals. From all aspects the trucking procedures and climatic conditions were considered ideal.

The variation and generally high percentages of exclusions in this experiment suggest that it is important to pay attention to the stress associated with transport and the preparation and handling of animals in the preloading period. Further interaction with prior trucking and handling experience may also contribute as reported by Stockman (2011) in relation to trucking of naïve versus habituated cattle. Results from replicate 2 indicated that it was possible to prepare, load and transport animals over considerable distances and up to 36 hours without resulting in excessively high ultiumate pH or meat colours.

Finally blood glucose was related negatively to eating quality which raises the possibility of this measurement being researched as another potential input for MSA grading, particularly in carcasses which have been subjected to pre-slaughter stresses. However the usefulness of glycogen as a predictor of eating was rather low when the reduced data set of carcasses which had achieved the thresholds of ribfat, ultimate pH and meat colour was examined. This would suggest that glycogen had a rather limited role as a potential indicator of quality particularly since there would be a cost in implementing such a technique into abattoirs with only a small increase in precision.

There remains a desire to find a practical individual animal measure of stress that can be applied at slaughter and incorporated as a threshold value or within the MSA model prediction. If this was achieved the grading criteria could be focused directly on the individual carcass with less concern in regard to generalized pre-slaughter thresholds such as time from property to slaughter or conditions regarding transport and rest breaks.

5 Recommendations

The first recommendation was that increasing the trucking time for cattle from 12 to 36 hours before an overnight rest in lairage and then slaughter was unlikely to impact negatively on eating quality.

However it should be noted that there was a large variation between replicates in the proportions of cattle that did not achieve the minimum ribfat, an ultimate pH < 5.7 and a meat colour score < 3. As there were no consistent treatment differences in the percentages of exclusions due to trucking treatments this would suggest replicate differences were due to a combination of cattle, prior experience and training of cattle, handling and nutrition could have contributed to the variation between replicates.

The second recommendation was that the MSA data base be monitored for the percentage of exclusions due to ultimate pH and meat colour and to a lesser extent ribfat in cattle consigned to MSA which are trucked more than 12 hours. This will quantify the extent of carcasses which fail MSA in cattle trucked long distances.

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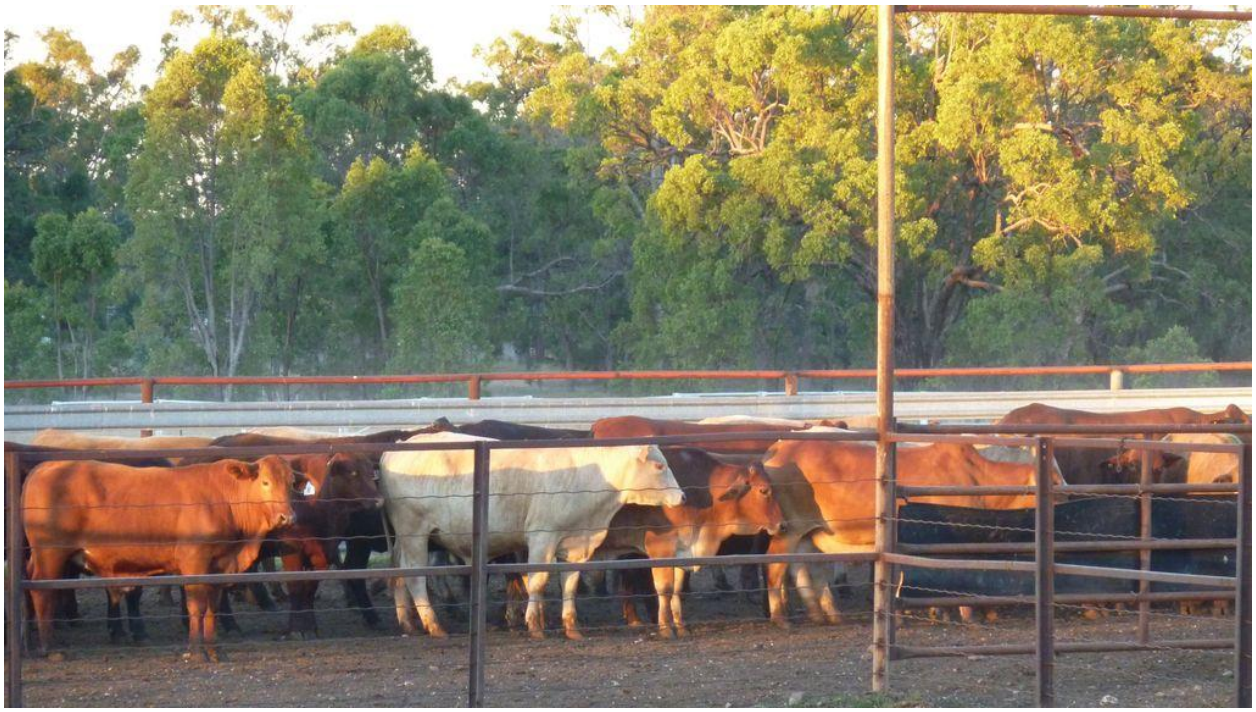
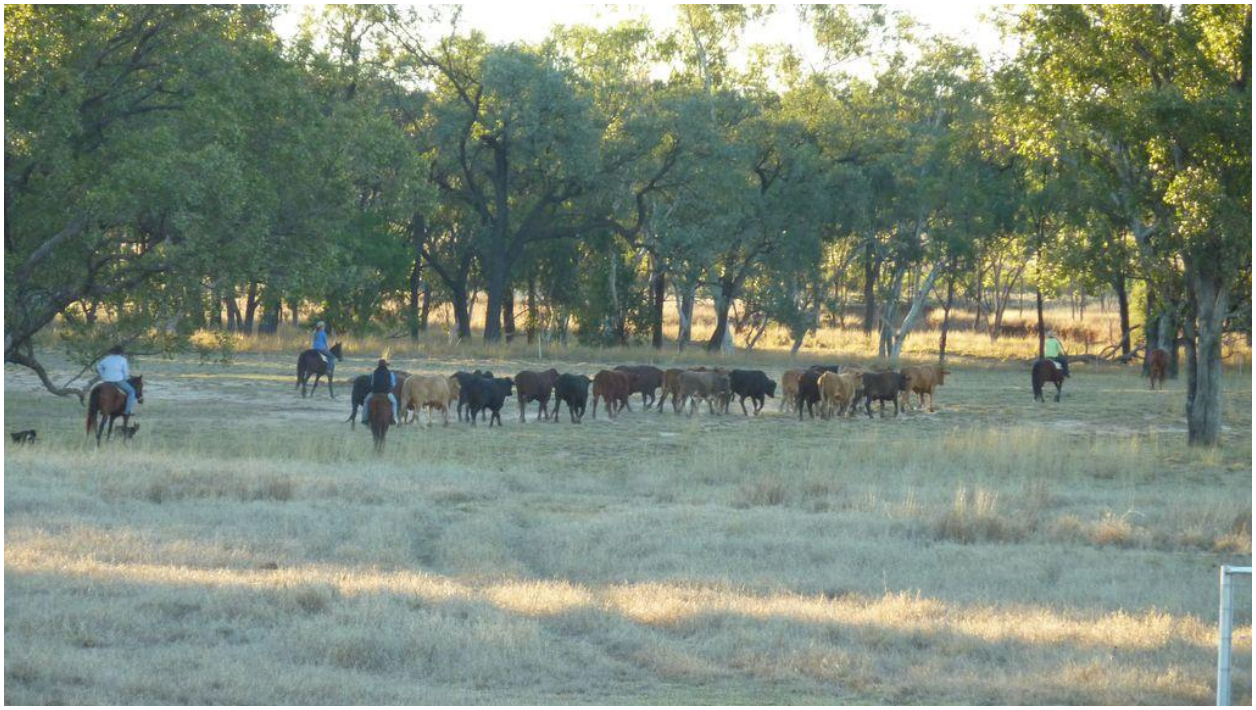
8 Appendix 1Photographs of the experiment in progress


BRECKON



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