Final report

MSA Saleyard Pathway Evaluation & Feeding Options

Project code: P.PSH.0513

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Date published:

PUBLISHED BY
Meat & Livestock Australia Limited
PO Box 1961
NORTH SYDNEY NSW 2059

This is an MLA Donor Company funded project.

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

Abstract

The project objective was to quantify the eating quality impact of alternative cattle marketing practices with four saleyard protocols compared to a direct to slaughter control, with cattle sourced from New South Wales (NSW) (Rep 1) and Victoria (Rep 2). This research conducted 2 replicates using the same experimental design. Each replicate utilised 5 treatment groups of mixed sex cattle (n = 120) from 4 properties of origin (2 properties supplied steers and 2 supplied heifers) of different breeds that were balanced across treatments. The control treatment groups (n = 24, six from each property, never mixed) were directly consigned to the abattoir (control 24hr), while the cattle for the other 4 treatment groups all were penned with their property contemporary group during an auction.

The saleyard treatments were

- extended Meat Standards Australia (MSA) saleyard pathway (48 HR, not mixed & water only),
- 72 hours (72 HR, 1 day on feed at saleyard),
- 7 days (7 DAY, 6 days on feed at saleyard) and,
- 14 days (14 DAY, 13 days on feed at saleyard).

The 72 HR, 7 DAY and 14 DAY groups were mixed contemporary groups post-sale with access to a feed ration and potable water.

The impact of treatment on tenderness, juiciness, flavour, overall liking and meat quality score (MQ4) were measured using untrained consumers for grilled *M. longissimus lumborum* (STR045) and *M. semitendinosus* (EYE075) aged for 7 days. The effect of muscle, treatment group and their interaction were analysed in a linear mixed effect model in R (Statistical Software) with animal within producer used as the random term for both replications. Further, the combined data (Rep 1 and Rep 2) was analysed in a linear mixed model with animal within producer and replication as the random term. Carcase traits were also included in the models as covariates and interacted with cut but were removed if they were non-significant.

For Rep 1, the 7 DAY and 14 DAY refeeding treatments had adverse effects on tenderness, juiciness, flavour and overall liking when compared to the directly consigned treatment ($P < 0.05$). The 48 HR treatment also had reduced juiciness, flavour and overall liking ($P < 0.05$), with no significant difference observed in MQ4 and tenderness when compared to the control 24hr group. No significant differences in consumer eating quality factors were observed between the directly consigned group and the 72 HR treatment. Further, the liveweights of the cattle in the 48 HR and 72 HR treatments were lower than their on-farm weights but the 7 DAY and 14 DAY re-fed cattle did not differ. The average hot standard carcase weight (kg) of the cattle in the 48 HR (266.3), 72 HR (272.9) and 7 DAY (273.1) were lower compared to the 14 DAY (280.0) and the direct kill group (280.4). Dressing % relative to the farm exit weight appears to be greatest for the direct 24hr group (52.74%) from other treatments. The highest average dressing % in relation to the final liveweight (54.87%) is the unfed post sale group (48 HR) which also has the lowest average dressing % relative to the farm exit weight (51.81%) reflecting reduced gut fill at saleyard exit and reduced carcase weight relative to farm weight. Refeeding cattle at the saleyard up to 6 days (7 DAY) and 13 days (14 DAY) post auction was not found to improve eating quality of beef, however, extending the saleyard pathway

out to 72 HR (1 day on feed) was not detrimental to eating quality in this cohort. Hump height was the only carcase characteristic that significantly impacted consumer sensory eating quality attributes in Rep 1.

For Rep 2, and to the contrary, the 14 DAY re-fed treatment had significantly improved consumer scores (3.63 points) in the flavour ($P < 0.001$) compared to the control 24hr group. No significant differences were found between control and other treatment groups across all sensory attributes scores. The direct-to-slaughter treatment (control 24hr) produced the highest hot standard carcase weight relative to farm weight result in addition to superior MSA compliance. Additionally, although not statistically significant, the dispatch that took place immediately after sale (48 hours) without feeding led to a decrease in carcase weight and an improvement in final weight as compared to the direct 24-hour group. Any period of feeding improved liveweight and hot standard carcase weight but there was no consistent relationship to days on feed. Dressing % relative to the farm exit weight appears to be similar for all treatment groups. The highest average dressing % in relation to the sale yard exit liveweight was found for 72 HR, 7 DAY and 14 DAY treatment groups compared to the direct 24hr and 48 HR treatments. Ossification score was the only carcase characteristic that significantly impacted tenderness in Rep 2.

When both replicates were combined, the 72 HR treatment had improved consumer scores across all sensory attributes of MQ4, tenderness, juiciness, flavour and overall liking for both the STR045 and EYE075, compared to the controls, although not significantly higher than the other treatment groups. It implies from these findings that the MSA saleyard method could be extended out to 48 hours for the duration between the original property and slaughter, without mixing or feeding. Furthermore, the results indicate with a one-day high-energy feed offered, cattle can handle up to 72 hours between original property and slaughter without negatively impacting eating quality. In this scenario, the environment, pen size and the quality and quantity of feed delivered needs to be tightly controlled, which is not possible. Further research is needed to evaluate the impact of mob size on the impact of mixing and ration quality on outcomes. Given the inconsistent meat quality results between replicates and the possibility of refeeding cattle with variable results, which could lower their eating quality score from 6 to 9 points, all saleyard cattle, regardless of pathway, should be subject to the 5 point MQ4 score prediction penalty on all cut x cook combinations as per the current MSA Standards.

Executive summary

Background

This project is a joint initiative between the Regional Livestock Exchange (RLX) Operating Company, and Meat and Livestock Australia (MLA) aimed to increase the number of cattle eligible for the MSA program. Currently, less than 1% of the MSA graded cattle are consigned via the current MSA Saleyard Pathway. This is largely due to the pathway not being commercially viable based on the regulated timeframes (36 hours from farm to slaughter via saleyards). This results in cattle being ineligible for MSA grading, despite having the potential to meet MSA grading criteria. Increasing the number of cattle currently consigned through saleyards by 10% to be MSA eligible could result in a return of $15.7M per annum to industry. New and improved pathways to maintain eating quality through the saleyard/exchange systems could substantially increase the throughput of cattle through saleyards being MSA eligible.

There has been work undertaken in this area in the past (Warriss, 1990, Ferguson et al., 2007b, Warner et al., 1998, Butchers et al., 1998, Ferguson et al., 2007a), however, there were some confounding findings such as adverse weather events, nutrition, vendors, transport time which resulted in no quantifiable changes to the current MSA pathway. The previous MSA research also did not include pathways beyond 36 hours, other than (Ferguson et al., 2007b), which is of great interest for various other consignment pathways within MSA, including the boat transport pathway (Loudon et al., 2019).

The project objective is to quantify the eating quality impact of alternative cattle marketing practices with four saleyard protocols compared to a direct from farm to slaughter control. Within the project, the first and second replicates were conducted in New South Wales (NSW) and Victoria (VIC), Australia, respectively, utilising the same experimental design. 120 head of cattle were sourced from 4 different properties (n=30 hd per property) across the Central West and New England regions of New South Wales, managed through the Tamworth Regional Livestock Exchange and then processed in Scone for replication 1 (Rep 1). Likewise, for replication 2 (Rep 2) 120 head of cattle from 4 different properties (n=30 hd per property) across Victoria, were managed through the NVLX Barnawartha facility and then processed in Wagga Wagga. For each replicate, 60 heifers and 60 steers were sourced from 4 farms (2 farms provided each gender) and allocated randomly to a control and 4 saleyard protocols including; trucking directly after sale relative to feeding a moderate energy ration approximately 14% crude protein, min 9.8 to 10.8 MJ ME/kg and >40% neutral detergent fibre (NDF) for an extra day, one week or two weeks prior to slaughter. Striploin and eye round primals were collected after MSA grading from all cattle with MSA consumer samples fabricated from each. Sensory testing was conducted on grill samples aged for 7 days post-mortem. The purpose of this study is to identify the impact of saleyard pathways on eating quality. It is intended that the results of this study be used to identify key points in the marketing pathways where control measures may be targeted to improve eating quality through re-feeding and resting regimes after stress events, or adjustments that may be required for eating quality predictions.

Objectives

The desired outcome of this project is to commercially validate the existing MSA saleyard pathway, an extended pathway, to enable an increase in the growth of MSA graded cattle that can achieve the necessary eating quality compliance. The project outcomes will enable the MSA pathways committee to approve further MSA pathways if the data provides adequate validation. The project may also determine what further research may be undertaken to explore further possible pathways or best practice.

The output will be to:

• Determine the viability of extended (48 HR and 72 HR) pathways as opposed to the current 36 hours (36 hrs) requirement.

• Feeding onsite pathway – re-establishing MSA eligibility after feeding saleyard cattle a high-quality feed post sale process (72 HR, 7 DAY and 14 DAY treatment groups).

• Pathway variations – variations to the above pathways including mixing sex as per mixing vendor and mixing on truck for delivery to processor.

The project will achieve the objectives listed above over 2 replicated trials consisting of 5 kill dates.

Methodology

- This research utilised 120 cattle from NSW (Rep 1) and 120 cattle from Victoria (Rep 2). For each of the replicates, mixed sex cattle of different breeds came from 4 properties of origin and were balanced across 5 treatment groups.
- The direct consignment (control) treatment group consisted of 24 head (6 from each property) in each replicate. Six cattle were directly consigned from each property to the abattoir and were maintained in their contemporary group till slaughter (not mixed), while the cattle for the other 4 treatment groups (n=24 from each property) were all penned within their property contemporary group during a saleyard auction.
- The saleyard 48 HR group was an extension of the current MSA Saleyard Pathway (36hrs) with cattle maintained in contemporary groups and given access to potable water only for an additional 12 hours.
- The three re-fed treatments were i) Saleyard 72 HR treatment ii) Saleyard 7 DAY treatment and iii) Saleyard 14 DAY treatment. The re-fed treatments were mixed contemporary groups post-sale with access to potable water and a high-energy, high protein feed ration delivered into rubber belt bunks at the onsite feeding yards.
- At each kill for both replicates, the project cattle were processed in an unbroken kill order with the number of lots compliant with research criteria. For the control, 24hr and 48 HR kills, the 6 head from each supplier were presented as single lots of 6. For the subsequent kills for Rep 1 each treatment presented as a single lot of 24. While for Rep 2 each kill was managed as 2 lots, one being the 6 head with HGP and the second the 18 non implanted mixed consignment.
- The *M. longissimus thoracis et lumborum* (MSA muscle code STR045) from the striploin and the *M. semitendinosus* (MSA muscle code EYE075) from the eye round were collected. Grill steaks

(25mm thick) were prepared from the head end of the *M. semitendinosus* (EYE075) and the *M. longissimus thoracis et lumborum* (STR045) for consumer sensory testing using the same protocols and aged for 7 days.

- Consumer sensory data was processed using MSA modelling technology to create a combined eating quality score (MQ4) for each sample. The MQ4 score is calculated out of 100 using the weighted average of tenderness (0.3), juiciness (0.1), flavour (0.3) and overall liking (0.3) from the 10 consumers per muscle.
- The effect of muscle, treatment group and their interaction were analysed in a linear mixed effect model in R (statistical software) with animal within producer used as the random term. The combined data (Rep 1 and Rep 2) was also analysed in a linear mixed model with animal within producer within replication used as the random term.

Results/key findings

Combined analysis

- When the replicate data was combined, the 72 HR re-fed treatment had improved consumer scores compared to the control treatment across all sensory attributes of MQ4, tenderness, juiciness, flavour and overall liking for both the STR045 (Striploin) and EYE075 (Eye round) cuts, although not significantly different to the other treatment groups.
- The 48 HR, 7 DAY and 14 DAY re-fed treatments had a negative effect, though non-significant across all sensory attributes of MQ4, tenderness, juiciness, flavour and overall liking for both the STR045 and EYE075 compared to the direct consignment control treatment group.

Rep 1

- The average liveweights (kg) of the cattle in the 48 HR (484.9) and 72 HR (502.6) treatments were lower than their on-farm weights but the 7 DAY (517.9) and 14 DAY (537.0) re-fed cattle did not differ from their average on-farm weights (537.0).
- The average hot standard carcase weight (kg) of the cattle in the 48 HR (266.3), 72 HR (272.9) and 7 DAY (273.1) were lower compared to the 14 DAY (280.0) and the direct kill group (280.4).
- Dressing % relative to the farm exit weight appears to be greatest for the direct 24hr group (52.74) from other treatments. The highest average dressing % in relation to the saleyard exit liveweight (54.87%) is the unfed post sale group (48 HR) which also has the lowest average dressing % relative to the farm exit weight (51.81%) reflecting reduced gut fill at saleyard exit and reduced carcase weight relative to farm weight.
- The extension of current MSA pathway up to 48 HR (36 hours + 12 hours - water only) had significantly reduced the juiciness, flavour and overall likeness for both the STR045 and EYE075 ($P < 0.05$) compared to the direct consignment group while the MQ4 and tenderness did not significantly differ.

- The 72 HR treatment (1 day on feed) was not significantly different for any consumer sensory trait or MQ4 score to the direct consignment cattle.
- The 7 DAY and 14 DAY re-fed treatments had significantly reduced consumer scores across all sensory attributes of MQ4, tenderness, juiciness, flavour and overall liking for both the STR045 and EYE075 ($P < 0.05$) compared to the direct (control 24hr) and 72 HR treatment groups, except for juiciness where the 72 HR and 14 DAY treatments did not differ.
- The impact of re-feeding on beef eating quality showed a reduction of greater than 5 points across all consumer sensory traits in the 7 DAY and 14 DAY re-fed treatments compared to cattle that were directly consigned. Surprisingly, this adverse impact was not observed in the 72 HR treatment for any consumer sensory trait or MQ4 score, implying prolonged exposure to stress has greater impact on eating quality than an initial stress event for all the meat quality attributes in replicate 1.
- Cut had a significant impact on all consumer sensory eating quality attributes ($P < 0.001$) with the STR045 eating better than the EYE075. The interaction between cut and treatment was insignificant across all sensory attributes.
- Hump height was the only carcase characteristic found to significantly impact consumer sensory eating quality attributes.

Rep 2

The direct-to-slaughter treatment (control 24hr) produced the highest hot standard carcase weight relative to farm weight result in addition to superior MSA compliance. Additionally, although not statistically significant, the dispatch that took place immediately after sale (48 hours) without feeding led to a decrease in carcase weight and an improvement in final weight as compared to the direct 24-hour group. Any period of feeding improved liveweight and hot standard carcase weight but there was no consistent relationship to days on feed.

Dressing % relative to the farm exit weight appears to be similar for all treatment groups. The highest average dressing % in relation to the saleyard exit liveweight was found for 72 HR, 7 DAY and 14 DAY treatment groups compared to the direct 24hr and 48 HR treatments.

Only the 14 DAY re-fed treatment had significantly improved consumer scores in flavour for both the STR045 and EYE075 ($P < 0.05$) compared to the other treatment groups. No significant differences were found between the control and other treatment groups across all sensory attributes scores.

Benefits to industry

The research project provides essential data to quantify the eating quality impact of alternative cattle marketing practices with four saleyard protocols compared to a direct consignment from farm to slaughter control sourced from both New South Wales and Victoria. The results suggest that the MSA saleyard pathway could be increased up to 48 hours without mixing and providing water. Although the 72 HR pathway had a positive relationship with all eating quality attributes for both replications, the cattle were provided high-energy feed supplementation for 1 day. Apart from enhanced flavour in the 14 DAY treatment group in rep 2, the 7 DAY and 14 DAY re-fed treatments did not improve any of the other sensory characteristics of the beef. Additionally, it is advised that research is continued to determine why the treatment effects on the meat-eating quality attributes differed between the two replicates and is required to substantiate if there is any consistent feed and breed effect.

Future research and recommendations

Since the MQ4 and tenderness in the 48 HR group in Rep 1 were comparable to those of the direct consignment group and neither Rep 2 nor the combined trials significantly differed from the controls, the MSA saleyard pathway could be extended to a maximum of 48 hours following the current MSA saleyard pathway rules. The 48 HR pathway must involve no mixing of cattle.

Although no significant differences in consumer eating quality factors were observed between the directly consigned group and the 72-hour treatment group for each replicate and the combined analysis, it was determined there was insufficient evidence to support the adoption of the 72 hours with refeed pathway. Additionally, commercial implementation of a pathway including refeeding would require additional auditing and strict processes in place at saleyards to ensure the feed quality requirements were met, along with other factors such as frequency of feeding, pen size, suitable feeding equipment and feeder access. Another factor that could change the outcome is mob sizes that are mixed. In this experiment, mobs of 6 were mixed together however in commercial reality the mob sizes may be very uneven which could change group dynamics, stress responses and eating quality outcomes. This needs further research.

Given the inconsistent meat quality results between replicates, which could lower their eating quality score from 6 to 9 points, all saleyard cattle should be subject to the 5 point MQ4 score prediction penalty on cut x cook combinations.

The 7 DAY and 14 DAY groups in Rep 1, reduced all eating quality attributes higher than 5 points, thus it is not appropriate to practice these saleyard pathways. The large variation in 7 DAY and 14 DAY groups in Rep 1 and Rep 2 means more research is required to understand impacts of these extended refeeding intervals.

Attention is drawn to the need to ensure sufficient time between slaughter and grading along with stimulation to be optimised to enable correct pH/temperature declines and a genuine ultimate pH reading at grading.

Also of interest, is the consistent difference in ultimate pH between the *M. longissimus lumborum* (grading muscle) and the *M. semitendinosus*. Consistent pH differences between muscles within a carcase are considered highly likely to impact ultimate eating quality, and in particular flavour development, through interactions with muscle structure and composition, packaging, ageing, and cooking. A better understanding of these relationships could have important ramifications for future MSA grading.

Additionally, it is clear that more research is needed to identify and quantify the management techniques, stress levels in different treatment groups and causes of the different treatment outcomes for the replicates, allowing for the widespread adoption of a particular method/s for enhancing meat eating quality.

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1. Background

Consumer acceptance of beef eating quality is essential to the prosperity of the industry, driving consumption, product repurchase and willingness to pay in Australia's primary markets (Shackelford et al., 2001, Feuz et al., 2004, Špehar et al., 2008, Bonny et al., 2017, Polkinghorne and Thompson, 2010). Eating quality is becoming progressively important in the competitive protein marketplace as consumers are increasingly educated and meticulous in their selection (Henchion et al., 2014). This is particularly important given the high price point of beef in the broader meat market due to longer product turnover, increased supply chain costs and high costs of production. At the point of consumption, intrinsic eating quality is determined by the levels of tenderness, juiciness and flavour the consumer experiences allowing a formulated measure of overall liking (Pethick et al., 2010). Historically tenderness has had the largest impact on the consumers evaluation of eating quality at consumption (Smart, 1994, Egan et al., 2001, Bonny et al., 2017). Beef that consistently delivers acceptable tenderness levels has been found to significantly increase consumer satisfaction and repurchase intent (Miller et al., 2001). Due to the improvement of beef tenderness across the industry in the past 30 years and changing consumer preferences identified using discriminate analyses, Meat Standards Australia (MSA) has allocated equal weighting to flavour and tenderness in their determination of a consumers sensory eating quality score (MQ4 score) (Bonny et al., 2017). A high level of eating quality and product consistency is essential in providing favourable eating experiences to the consumer to attract product repurchase and price premiums for Australian beef.

The variability in production systems across the Australian beef industry, reflecting differences in climate, pasture, industry infrastructure and proximity to markets, dictates the need for multiple marketing pathways in the supply chain including the demand for intermediatory steps between production and processing. Traditional saleyards auctions are still most common selling method of cattle in the Australian supply chain offering producers the ability to sell varying numbers and classes of cattle, contract agents to sell cattle on their behalf and providing competitive pricing (Driedonks et al., 2005). The pathway accounts for approximately 40% of all cattle sales (Iglesias and East, 2015) and according to Meat and Livestock Australia (2022) saleyard survey results, 3.7m cattle were sold via saleyards in the 2022 financial year. Nevertheless, its popularity has been declining over the past 2 decades (Riley et al., 2000). This is most likely due to increasing numbers of cattle sold through online auctions and increasing direct sales for feeder and finished cattle. Declining saleyard numbers may be due to growing awareness of the effects of animal stress on liveweight, eating quality, and production parameters, as well as refusal to pay commission to agents, inability to set a minimum price, and availability of these markets. Concerns regarding the impact of increased stressors and stimulation in the selling environment of intermediatory supply chain steps on eating quality and production has led to a higher proportion of cattle being sold through direct consignment pathway (Ferguson et al., 2007b). The implications of additional stressors associated with the saleyard marketing pathway has resulted in the MSA Standards defining additional supply chain rules as well as a 5 point eating quality adjustment on all cuts (Watson et al., 2008).

Exposure to stressors associated with a new environment is unavoidable across the beef supply chain as cattle are marketed from farm to slaughter, which is likely to increase with the implementation of intermediate steps in the supply chain such as saleyards and lairage (Ferguson et al., 2007b). Principle stressors associated with exposure to new environments include the mixing and association of cattle from different vendors, increased time off feed and additional human handling and stimulation (Loudon et al., 2019). Acute and chronic production stress has been found to adversely impact meat quality attributes including tenderness and ultimate pH (pH_u) (Knowles, 1999, Ferguson and Warner, 2008). The extent of the stress response and impact on performance is impacted by the duration and intensity of the event triggering the response (Ferguson et al., 2001). Preslaughter stress impacts tenderness in ruminants through its association with dark cutting and directly through a mechanism independent of ultimate pH (pH_u), however the mechanism causing increased toughness is still unclear (Grayson et al., 2016, Warner et al., 2007). Preslaughter stress has previously been found to reduce consumer sensory tenderness scores by 4.4 points (Warner et al., 2007). Thus, the impact of elevating stress responses in cattle through intermediate supply chain steps can negatively impact the eating experience of the consumer.

There is evidence that a resting or refeeding period prior to slaughter may assist is dissipating adverse eating quality effects caused by stress exposure throughout the supply chain (Knee et al., 2007, Loudon et al., 2019, Wythes et al., 1988). The re-feeding regime is dependent on the intensity of the stress event as muscle glycogen is rapidly depleted during stress (Terlouw et al., 2021), however, slow to replenish (Tarrant, 1989). Knee et al. (2007) determined that re-feeding regimes are required to be a minimum of 1 to 3 weeks long to effectively re-establish muscle glycogen levels after animals are subjected to stress. Further, cattle take approximately 3 to 4 days to re-establish their social hierarchy after mixing of contemporary groups has occurred along the supply chain (Doyle and Moran, 2015). Limited research has investigated the effect of resting cattle for extended time periods (>24 hours) on eating quality. Loudon et al. (2019) found paddock resting cattle 2 weeks after marketing through the saleyard pathway resulted in reduced biological stress markers at slaughter, likely to result in positive eating quality ramifications. Shorter resting periods of 1 and 3 days have also been found to improve tenderness of steers and cows post transport, with the greatest eating quality improvement observed in undisturbed resting treatments (Wythes et al., 1988). Therefore, it is possible to improve eating quality attributes of cattle through re-feeding and resting regimes after stress events.

The MSA Standards define pathway rules for both direct and saleyard consignment to mitigate the adverse eating quality impacts of stress. Both pathways require cattle to reside on property for 30 days prior to dispatch for slaughter (Pethick, 2006) to lessen new environment stressors and maintain muscle glycogen levels. Further, directly consigned cattle are required to be slaughtered within a 48 hour time period from leaving the property. This time period is less when cattle are consigned through a the MSA saleyard pathway, which is 36 hours from leaving the farm to slaughter (Pethick, 2006). Meeting these requirements can be commercially unachievable for producers due to the extensivity of Australian production systems with time between farm and slaughter typically ranging from 2 to 5 days (Wythes and Shorthose, 1984). Therefore, the aim of this experiment was to determine the impact of marketing method on eating quality and if refeeding a high energy ration could increase consumer eating quality. It was hypothesised that 14 days re-feeding of a high energy ration would dissipate any adverse eating quality effects from saleyard exposure and thus this treatment would have the same consumer palatability as the directly consigned pathway. Further, 72 HR and 7 DAY re-feeding pathways would not be long enough to overcome any negative eating quality impacts and thus would have reduced eating quality compared to the directly consigned pathway, but equal to that of the current MSA saleyard pathway.

Therefore, the research design sought to effectively compare four alternative saleyard management pathways to a direct to slaughter pathway, ranging in time to slaughter and re-feeding, as well as accounting for other factors such as mixing of cattle.

2. Objectives

This project is a joint initiative between the Regional Livestock Exchange (RLX) Operating Company, and MLA aimed to increase the number of cattle eligible for the MSA grading.

Currently, less than 1% of the MSA graded cattle are submitted via the current MSA Saleyard Pathway. This is largely due to the pathway not being commercially applicable or viable based on the regulated timeframes. The number of cattle that pass through saleyards that are eligible for MSA might be substantially increased by developing new and better techniques to monitor or understand the eating quality that is consumed through the saleyard/exchange systems

There has been work undertaken in this area in the past (Warriss, 1990, Ferguson et al., 2007b, Warner et al., 1998, Butchers et al., 1998, Ferguson et al., 2007a), however, there were some confounding findings such as adverse weather events, nutrition, vendors, transport time which resulted in no quantifiable changes to the current MSA pathway. The previous MSA research also did not include pathways beyond 36 hours, other than (Ferguson et al., 2007b), which is of great interest for various other consignment pathways within MSA, including the boat transport pathway (Loudon et al., 2019)

The desired outcome of this project is to commercially validate, an extended pathway and feeding pathways, to enable an increase in the growth of MSA graded cattle that can achieve the necessary eating quality compliance. The project outcomes will enable the MSA pathways committee to approve further MSA pathways if the data provides adequate validation. The project may also determine what further research may be undertaken to explore further possible pathways or best practice.

The output will be to:

- Determine the viability of extended (48 hours, 72 hours, 7 days and 14 days through saleyard) pathways.
- Feeding onsite pathway – maintaining MSA eligibility after feeding saleyard cattle a high-quality feed post sale process (72 hours, 7 days and 14 days treatment groups).
- Pathway variations – variations to the above pathways including mixing of different vendor groups prior to dispatch to processor.

The project aims to l achieve the objectives listed above over 2 replicated trials consisting of 5 treatments each.

3. Methodology

This study was approved by the University of New England Animal Ethics Committee (Approval No. AEC193-207) and was compliant with the Animal Research Act 1985 (as amended) in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (NHMRC, 2013). In addition, the sensory evaluation for each replicate was conducted with the approval of the University of New England's (UNE) human ethics committee (HE17-253) for replicate 1 and Charles Sturt University human research ethics committee for replicate 2 (H23526).

3.1 Principles and Experimental design

This research design aimed to represent Australian commercial supply chain conditions in order to provide industry-relevant recommendations on the effect of different marketing methods on the eating quality of beef cattle. This research utilised 120 cattle from the New South Wales (NSW) (Rep 1) and 120 cattle from Victoria (VIC) (Rep 2). For each of the replicates, five treatment groups totalling 120 head of mixed sex cattle of different breeds were balanced across treatment groups from four different properties (Fig. 1). The treatments as summarised below encompassed both mixed and non-mixed groups and alternative lengths of saleyard feeding for those groups held beyond the day of sale. The treatments were:

- Direct consignment (control 24hr): Direct consignment from the source property to abattoir with cattle to arrive on the evening of property despatch and be slaughtered the following morning (Control). Each source group to be maintained as an individual group in lairage and to slaughter.
- Saleyard 48hr treatment (48 HR): Trucking from property to saleyard the afternoon prior to sale with cattle retained in their source groups from farm, within the selling facility, during trucking to the abattoir and in lairage prior to slaughter. Cattle to have unlimited access to water but not fed at the selling facility and trucked to the abattoir at 16:00 Australian eastern standard time (AEST) post sale.
- Saleyard 72hr treatment (72 HR): Trucking from property to saleyard the afternoon prior to sale with cattle retained in their source groups from farm and during the sale, then mixed with the other three supplier groups immediately post sale and placed on feed for 24 hours prior to trucking to the abattoir at 16:00 (AEST) the day after the sale.
- Saleyard 7-day treatment (7 DAY): Trucking from property to saleyard the afternoon prior to sale with cattle retained in their source groups from farm and during the sale then mixed with the other three supplier groups immediately post sale and placed on feed for 6 days prior to trucking to the abattoir one week after arrival at the selling facility.
- Saleyard 14-day treatment (14 DAY): Trucking from property to saleyard the afternoon prior to sale with cattle retained in their source groups from farm and during the sale then mixed with the other three supplier groups immediately post sale and placed on feed for 13 days prior to trucking to the abattoir two weeks after arrival at the selling facility.

These treatments were designed to evaluate practices that could be commercially provided by the selling centre operator. The first treatment follows standard procedure, which calls for immediate post-sale slaughter within a 48-hour timeframe from farm to slaughter, to assess an extended MSA

Saleyard pathway. In the second treatment, a buyer or commission appears as they acquire small mixed-sex groups, assemble them after the sale, and provide a tailored diet. In order to make room for cattle sold in the following weeks, the 7 DAY and 14 DAY treatments prolong the feeding period and permit transport one day ahead of the scheduled sale day.

This feeding service could potentially be a commercial service for clients to spread kill dates, gain MSA eligibility or potentially add weight and or reduce stress. To constrain animal numbers and related expenses, the design specified an even distribution of sex with 30 steers sourced from 2 suppliers and 30 heifers from a further 2. To facilitate management all cattle were randomly allocated individual trial eartags with colour designating the treatment and sequential blocks (1 – 30, 31 – 60, 61 – 90 and 91 – 120) of numbers indicating the supplier. This in turn aggregated six head from each supplier within each of the 5 treatments processed on separate dates establishing five 24 head treatment comparisons. It was elected to impose a common time of drafting and despatch from each supplier rather than a common slaughter date, the potential seasonal effects at farm level being of higher concern than within abattoir variation.

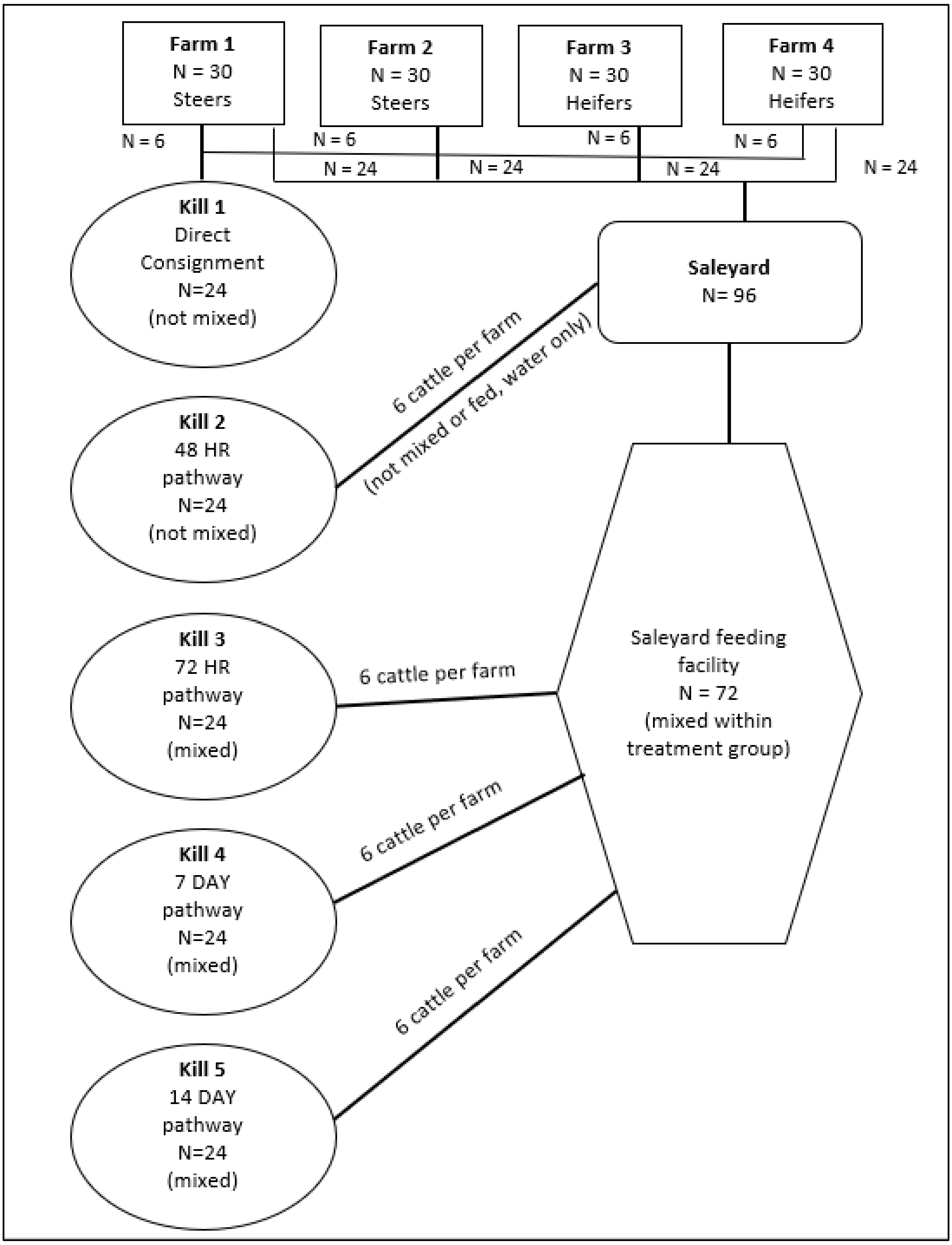

Figure 1. Trial diagram of timeline methodology for slaughter, resting and re-feeding intervals

3.2 Cattle selection for replicate 1 (Rep 1) and replicate 2 (Rep 2)

To ensure commercial relevance, the cattle specifications were for all cattle to be within MSA specification at the on-farm stage and expected to meet local market weights and fatness, aligning to market requirements and better end MSA grade outcomes.

3.2.1 Property and cattle

Each of the two replicates (Rep 1 and Rep 2) of the study had 120 animals from various Australian locations; each replication included five treatment groups of mixed-sex cattle from four distinct properties.

Rep 1 steers were sourced from two properties and heifers from the remaining 2 properties to employ a mixed sex trial design. The cattle were predominantly British and European breeds or crossbreds grown and finished on pasture with no hormone growth promotants (HGPs). The Tamworth Regional Livestock Exchange saleyard was selected for the study due to its capacity for cattle refeeding. For Rep 2, three groups were sourced from separate cattle mobs on two properties in the Milawa area of Victoria with 2 being from pasture with some supplementary feeding and the other from a feedlot group, but not eligible for grain-fed status. These cattle had not been treated with HGPs, whereas the fourth group had been implanted with HGPs. This property was a small feedlot facility located in the Yarrawonga region, but not eligible for grain-fed status.

Cattle were initially balanced on farm for weight and breed type rejecting animals with extreme differences leaving a total of thirty for the experiment. On farms, cattle were sequentially drafted into those directly consigned (n = 6, numbers 3, 8, 13, 18, 23 and 28 respectively through the race) and those transported to the saleyards (n = 24).

3.2.2 Allocation of animals to treatment group and marketing pathways

All cattle from each property were loaded onto a semi-trailer with two decks. For practical and economic reasons, the six direct (control 24hr) and the twenty-four other mixed cattle groups from each farm were loaded on a common truck but penned separately for immediate transfer to the facility. On arrival they were unloaded with the six direct head groups penned separately in pens adjacent to the double deck loading ramp. The 24 head groups were penned in larger pens closer to the designated weighing and drafting facility. All pens were within the roofed area, soft floored and had unrestricted water access.

At the abattoir, the directly consigned cattle from each property were placed in undercover lairage yards overnight in their contemporary groups and slaughtered the following morning. At the saleyard, cattle were penned overnight in their contemporary groups with access to water. The following morning, cattle were sequentially drafted in their property contemporary groups into four treatments, i) Saleyard 48hr treatment (48 HR), ii) 72 HR re-feeding (72 HR, 1 day on feed), iii) Saleyard 7-day treatment (7 DAY, 6 days on feed) and iv) 14 DAY re-feeding post sale (14 DAY, 13 days on feed). The 48 HR treatment groups were held overnight in the covered sale pens in their property contemporary groups prior to trucking the following morning for a same day kill. For the remaining 72 HR, 7 DAY and 14 DAY groups that were to be mixed post sale, the final grouping was visually conveyed by utilising Green (72 HR), Yellow (7 DAY) and Red (14 DAY) tag colours for all farms. Each property contemporary group was penned within their treatment group (n = 6) in a randomised pattern within the selling environment adjacent to other groups that were sold.

To facilitate management, all cattle were allocated individual trial eartags with colour designating the treatment and sequential blocks (1 – 30, 31 – 60, 61 – 90 and 91 – 120) of numbers indicating the supplier and ensuring a unique trial-specific visual ID linked to the NLIS printed code and RFID number for each replicate. Each animal was weighed, then scanned, eartag checked and data including time recorded.

The tag colour and number allocation are summarised in Table 1. Unique farm-related eartag colours were assigned to the direct and immediate post-sale (48 HR) treatment groups to provide a strong visual cue that "colour represented a group that must be kept separate and never mixed at the saleyard or abattoir". The tags were numbered and arranged in draft order prior to the farm visit to align with the allocation in Table 1.

Table 1. Trial eartag colour and number allocation

Head	Eartag Range	Colour	Property	Treatment	Notes
6	1 to 6	Orange	A	**Direct**	New Orange Tags to replace existing ex property
6	7 to 30	Purple		48 Hrs	These 24 head mixed for trucking to RLX as a single lot
6		Green		72 Hrs	
6		Yellow		7 Days	
6		Red		14 Days	
6	31 to 36	White	B	**Direct**	New White Tags to replace existing ex property
6	37 to 60	Pink		48 Hrs	These 24 head mixed for trucking to RLX as a single lot
6		Green		72 Hrs	
6		Yellow		7 Days	
6		Red		14 Days	
6	61 to 66	Blue	C	**Direct**	New Blue Tags to replace existing ex property
6	67 to 90	Orange		48 Hrs	These 24 head mixed for trucking to RLX as a single lot
6		Green		72 Hrs	
6		Yellow		7 Days	
6		Red		14 Days	
6	91 to 96	Black	D	**Direct**	New Black Tags to replace existing ex property
6	97 to 120	White		48 Hrs	These 24 head mixed for trucking to RLX as a single lot
6		Green		72 Hrs	
6		Yellow		7 Days	
6		Red		14 Days	

As shown in Table 1, five tag colours were sequentially applied. One designating the direct to abattoir control placed in a separate column to clearly indicate that these six head were to be drafted to a separate pen relative to the other 24 with 4 other coloured tags. Rejected cattle were not recorded and were drafted a third way. Farm drafting and truck loading times were recorded on the farm record sheets (Appendix I).

The trucking distance between the properties and abattoirs ranged from 150 to 320 km for Rep 1 and ~160 km for Rep 2 with transport time approximately 2 hours.

3.2.2.1 Sale and post-sale day procedures

The research cattle remained in the sale pens throughout the actual sale with normal agent and buyer activity in close proximity including substantial cattle and people movement in the vicinity.

Each of the four 48 HR unmixed treatment group pens of 6 were returned to the weighing and drafting facility after the sale, weighed and scanned individually with the weights manually recorded on the recording sheets provided for each replicate.

After completion of weighing, each group of 6 were transferred to an individual loadout pen adjacent to the ramp. These were loaded for transport to the designated abattoir. The same truck and driver were used for all cattle movements, as far as practically possible, to ensure uniform handling and trucking exposure. A target time for loading at 16:00 (AEST) was agreed for all groups. The truck crate was of excellent design with smooth sides, no protrusions that could cause bruising and having 4 uniform sized pens suitable to maintain segregation of 4 groups of 6 head. For all movements from the saleyard to the abattoir, Rep 1 cattle travelled 150 to 320 km and Rep 2 cattle travelled 141 km.

As external cattle movements allowed post sale, the green tag (72 HR) cattle pens were boxed and the resulting 24 head transferred as a single mob to an external larger dirt floored pen, but close to the scale and drafting facilities used previously. The yellow tag (7 DAY) groups were also boxed and transferred to a second external pen adjacent to the green tag group as were the red tag (14 DAY) group.

All groups were weighed and scanned on their nominated dispatch day, typically around two hours prior to loading. For the mixed groups in Rep 2, the 6 HGP treated cattle identified by eartag number being above 90, were drafted off and penned together during trucking to facilitate processor allocation to HGP and non HGP kill timing.

The three re-fed treatment groups (72 HR, 7 DAY and 14 DAY pathways) were placed in 3 separate feedlot style feed yards with access to a high quality ration mixed on site. The ration for Rep 1 was 35% concentrate pellets, 30% lucerne hay (chopped), 20% white cotton seed and 15% oaten hay (chopped) formulated to 17.05% CP and 10.2 MJ/kg DM (Table 2). For Rep 2, roughly 40% pasture and cereal hay chopped and mixed with 60% concentrate meal provided by Reid Stockfeeds prior to feeding. The concentrate meal was formulated to provide a safe composition for cattle that had come from pasture feeding while also providing higher energy and protein levels to enable weight gain. Table 3 displays the ingredients used in the mix and the nutritional outcome in Rep 2. Equipment and facility limitations precluded precision in batching ration mixes and accurate recording of individual pen intake.

The feed ration was mixed and delivered ad libitum utilising a PTO driven mixing trailer behind a tractor every afternoon, with adjustments made by the feed team based on feed left in the trough. The 72 HR re-fed treatments were trucked roughly 48 hours after leaving the home property and slaughtered the following morning. The 7 DAY re-fed treatments were loaded in the afternoon of day 7 and transported to the abattoir where they were held overnight in lairage and slaughtered the following morning. This process was replicated the following week for the 14 DAY re-fed treatments which were transported to the abattoir on day 14.

Table 2. Feed quality laboratory analysis (Rep 1)

Test description	Units	Results
Metabolisable energy	MJ/kg DM	10.20
Crude protein (DUMAS method)	%	17.05
Crude fat	%	5.40
Dry matter	%	77.50
Digestible dry matter	%	57.00
Neutral detergent fibre	%	50.00
Acid detergent fibre	%	30.50
Inorganic ash	%	9.45
Note: DUMas method for calculating crude protein (Simonne et al., 1997)		

Table 3. Concentrate mix blended with hay and fed to 72 HR, 7 DAY and 14 DAY treatment groups for Rep 2

Commodity	KG per Tonne	Ration Analysis	
Barley	386.6		
Almond Hull	300	Metabolisable Energy	11.9
Canola Meal	125		
Lupins	100	% Crude Protein	14.20%
Maize	50		
Limestone	9.6	% NDF	25.80%
Bentonite	7		
Mag Sulphate	5	% STARCH	27.20%
Acid Buff	5		
Canola Oil	5		
Sodium BiCarb	3		
Salt	3		
Reid Dairy Premix	0.8		
Total	**100**		

All pens were fed ad-lib but daily bunk observations, mix weights, individual pen intakes and remaining feed were not recorded. While this adhered to commercial practice of a fixed daily feeding charge related to ad-lib hay feeding, a more detailed study of feeding periods combined with accurate measurement of ration composition and intake related to live and carcase weights is recommended to develop reliable guidance for commercial application.

3.3 Slaughter and primal collection

At each kill for both replicates, the project cattle were processed in an unbroken kill order with the number of lots compliant with research criteria. For the control 24hr and 48 HR kills, the 6 head from each supplier were presented as single lots of 6. For the subsequent kills for Rep 1 each treatment presented as a single lot of 24. While for Rep 2 each kill was managed as 2 lots, one being the 6 head with HGP and the second the 18 non implanted mixed consignment.

For both replicates, the following procedures were followed for each of the 5 research consignments. Slaughter was completed using captive bolt stunning followed by immediate exsanguination and dressing. The NLIS tag RFID code was read at the knocking box and recorded on the plant IT system following normal plant procedure. Abattoir body numbers were assigned post sticking and indicated by a printed number stuck to the hide until reaching the scale and having final carcase tickets attached. The trial visual eartag was also entered manually by abattoir personnel.

In addition, as a final backup to ensure failsafe, animal to carcase ID in the event of a misread or system issue, research personnel and an abattoir supervisor removed both the trial visual tag and the RFID/NLIS tag after the second leg position and placed them in consecutively numbered Ziplock bags to retain the ability to physically relate body number to both electronic and visual ID.

The RFID and NLIS electronic files and the manually recorded last 4 NLIS digits for each animal recorded at each farm were later crosschecked with those recorded at the saleyard and through the abattoir system, with the physical tags used to confirm the visual ID linkage to carcase number. Once all correlations were confirmed, and any discrepancies resolved, the physical tags were discarded, and abattoir body number utilised as the ongoing ID chain.

For Replicate 1, a grader recorded the ossification cold along with the remaining carcase characteristics at time of grading. This differed for Replicate 2, where a grader recorded hot ossification at the carcase scale immediately before the carcases were transported to the chiller after freeing the cube roll and removing the feather bones. As the feather bones on the chine were removed at this point hot ossification was needed to cover the possibility that ossification may reach the rib area.

The carcases were then conveyed to a chiller with a spray chill program activated to bring the sides down to the required temperature for boning the following day. MSA personnel conducted hourly pH temperature and pH measurements in the posterior of the *M. longissimus lumborum* (LD) muscle at the abattoir for 4 to 6 hours following slaughter. Five readings were taken on all carcases. These readings were then entered to document individual pH and temperature decline curves for all carcases.

On the day following each kill the research sides were opened (ribbed) at the 12^{th} rib and allowed to bloom for a minimum of 20 minutes prior to grading by MSA personnel (Rep 1 and 2 except the last kill for Rep 2 graded by company grader). Grading followed MSA protocols with hump height, MSA marbling, ossification (with reference to the hot readings where needed), rib fat depth, eye muscle area, meat colour, fat colour, pH and temperature measured on the left side exposed surface of the LD. Carcase weight, sex and HGP status were scanned from the carcase ticket and other data were entered into a data capture unit (DCU) and then exported as a grading file.

Prepared laminated Primal Cut tickets were then securely attached to the striploin and eye round primals with 150mm stainless steel pins. The primal tickets displayed a prominent Cut Up Developer (CUD) no., a sequential number assigned as a proxy for carcase number given they were prepared, printed on coloured paper, laminated and cut to individual tickets prior to the kills. Also displayed was the cut name and a unique 5 digit primal number utilised in later fabrication. An example pair of tickets are displayed in Fig. 2.

4	**4**
69561	**69560**
EYE ROUND	**STRIPLOIN**

Figure 2. Example of primal tickets for one carcase side.

After grading the left sides were marshalled by abattoir staff onto discrete rails within the holding chiller adjacent to the boning room and assigned a boning batch. When the research carcases were due to be boned, research and abattoir personnel were placed at strategic positions along the boning line to ensure tags were not removed and to assist in collection of the two cuts. Those placed adjacent to the striploin boners and slicers and those on the butt boning line monitored the cut removal and minor trimming ensuring that if a pin or tag was temporarily removed for safe knife access, it was placed securely back in the correct cut as soon as possible. The tagged primals were then wheeled to a vacuum packing station where the pin was removed and placed on a stainless-steel frame with the Primal Tag placed upwards on the fat face of the cut and vacuumed within the bag. The pins were counted to ensure all cuts had been retrieved and that no pins had been lost within the room.

Cut counts were then confirmed and the cuts placed in cartons and assigned research codes to identify the research product. The cuts were then passed to the chill tunnel and chilled for approximately 24 hours prior to pick up. Each primal was vacuum packed with their corresponding label and chilled at approximately 1°C during transportation.

In order to prepare sensory samples, Rep 1 samples were sent to the University of New England (UNE) in Armidale, while Rep 2 samples were sent to the Charles Sturt University (CSU) in Wagga Wagga.

3.4 Sample preparation

3.4.1 Primal processing to consumer sensory samples

As in all MSA sensory research projects, a design was established through discussion with industry users, the MSA team and reviewed by the MSA Pathways Committee. Once the parameters were agreed the design was implemented through use of the MSA Cut Up Developer (CUD) software (Appendix II). The CUD programs ensure linkage between nominated cattle groups, slaughter, boning detail Identification (ID) and subsequent fabricated consumer samples. The programs produce working files to support each step of the research activity and assign unique ID to animal>carcase>side>cut>muscle>muscle position>sample. After adjustment for any deviation as physically implemented these files are loaded to the MSA AUSBlue database. Further related data including the farm and saleyard recorded weights and the MSA grading files are also loaded to AUSBlue and connected to each sample as appropriate. A Master Group that relates all kills within saleyard trials and a Group ID that relates to this kill only is established.

The initial entries to create the CUD for the direct from farm control 24hr group. The second step expands to establish the number of sensory samples to be fabricated from each muscle, the position from which they are to be taken, the days of ageing and the cook method to be applied (Appendix III).

It is noted that a cooking method of LNK is displayed which is a workaround used to produce a sample label that allows “Link” (first consumer samples served) to be readily identified for sorting after sample vacuum packing. It is seen that two samples are designated for the striploin from the first and second anterior position and a single sample from the head of the eye round. All samples have a specified 7 day ageing period.

The third design step combines the previous data to create a data row for each sample and relates each to a CUD No. which is a placeholder for the actual abattoir body number once known. It is noted that the sensory samples are taken from the striploin A1 position and the Link from the A2 position in all cases to reduce potential muscle position variation and that an objective sample that can be accessed for Intramuscular Fat percentage (IMF%) or other laboratory study is specified by the “y” in the OBJ column (Appendix IV).

3.4.2 Application within abattoir

The Acquisition File was firstly generated to establish the link between CUD Body ID and the actual Abattoir Body number and to designate the Primal No. tickets to be pinned to each carcase with these aligned to the CUD Body ID. The working nature of the file is illustrated by the recording of Works Body No. in the AnimalID column due to the CUD ref being used as a Body No. to enable generation of CutUp files prior to the kill and actual Body No. being known (Appendix V). This file was also utilised to confirm the CUD No. and Primal No. on the laminated Primal Tickets that were pinned to the cuts on each body. The primal tickets were vacuum packed with the related primal as boned to retain individual primal cut ID to the source body and from Body No. to animal Visual Identification (VID) and Electronic Identification (EID). Once collected and chilled the cartons were transported to the laboratory for fabrication.

3.4.3 Meat laboratory sample fabrication

Further CutUp files produced from the CUD program provided work instruction, sample labels and recording relating to the fabrication and subsequent freezing of consumer samples. A portion of the CutUpFile that dictates all fabrication for the 2 primals that were collected from the CUD Ref 1 (Appendix VI). It will be seen that the ID is maintained by the Primal No. which was directly read from the laminated Primal Ticket placed within each primal as vacuum packed.

As the primal was removed from the carton and the bag opened the Primal Ticket was placed on a plastic serving tray with the full primal. At this point a pH reading was taken from each cut. All external fat and silverskin from the primal cut were removed and removed all but the designated muscle, in this project the *M. longissimus thoracis et lumborum* (MSA muscle code STR045) from the striploin and the *M. semitendinosus* (MSA muscle code EYE075) from the eye round.

After confirming that the muscle was aligned correctly on the tray (the anterior end of the STR045 or head end of the EYE075) to maintain a constant location to the right, the labels were removed from the CutupLabelFile and lightly attached to the edge of the tray aligned with the designated positions on the muscle. Where designated the IMF label was placed on the tray top edge. A horizontal mark was then made against each sample in the CutUpFile Check column to indicate the cut had been processed and the relevant labels removed. The labelled tray with Primal ticket and muscle was then passed to a fabricator. The fabricator utilised the labels on the tray edge as an instruction.

The muscle was then moved from the tray to a cutting jig set at 25mm spacing as dictated by MSA Grill protocols. The anterior face was “squared off” for the IMF sample and 25mm slices taken thereafter and laid out in order as cut to retain position and labelled with their position within muscle as per MSA sensory sample fabrication protocol (Watson et al., 2008). Depending on size, 2 to 3 sensory steaks (approximately 75 x 25 x 150 mm) were then trimmed from each slice in order until the required 5 per sample were produced. The 5 from the A1 position were then placed on the tray adjacent to the A1 grill (GRL) label and the second set of 5 from the A2 position placed on the tray next to the link (LNK) label for the STR045. Grill steaks 25mm thick were also prepared from the head end of the *M. semitendinosus* (EYE075) for consumer sensory testing using the same protocols as the *M. longissimus thoracis et lumborum* (STR045). Approximately 50g of meat, often the facing cut, were then placed adjacent to the IMF label and the tray moved to a packing position. A 250g steak was also collected and weighed before being frozen for shear force determination in Rep 1.

In accordance with MSA protocol each of the 5 steaks comprising a single sample were then wrapped in freezer wrap (to enable them to be broken apart while frozen) and placed within a 200 x 250mm vacuum bag with a unique identification reference number (EQSref) and sorted by cook type and ageing date into foam boxes. The foam boxes were held in a chiller at approximately 1^{o}C until the end of the 7 day ageing period, denoted by the DD/MM date code on the lower right of each sample label (Appendix VII). All samples were then spread single depth on trays or lids and placed within the freezer to achieve rapid and even freezing to approximately -20^{o}C.

Once frozen the samples were packed into foam boxes and remained in frozen storage until accessed for the next picking and posting step or required for laboratory testing. All planned samples were fabricated and the CutUpFile marked “Final” and uploaded to AUSBlue.

3.4.4 Objective meat quality measurements (Rep 1)

Objective measurements of shear force and cooking loss were conducted on samples aged for 7 days. The 250g sample from each primal frozen for shear force determination was defrosted, patted dry and weighed for determination of drip loss percentage. After weighing, samples were placed in plastic bags and cooked in a water bath at 80°C for 30 mins. Post cooking, samples were dried and weighed with cooking loss percentage expressed as the percentage of weight lost during cooking. Shear force was determined utilising a Lloyd Instruments LRX Materials Testing machine fitted with a 500N load cell (Perry et al., 2001). Six subsamples within each primal sample were cut, with the fibre orientation at right angles to the shearing surface.

IMF was determined by calculating the total fat content (%) present within the sample of meat. This includes both visible fat stored in adipocytes, visible to the naked eye at higher levels of IMF% and also fats held in the form of structural triglycerides within the cell wall (Harper and Pethick, 2004). Chemical IMF% was determined by Chloroform Soxhlet calibrated laboratory near infra-red (NIR) (Perry et al., 2001, Anderson et al., 2015, Stewart et al., 2021). This technique involves weighing the wet tissue in a tube, freezing it at -20^{o}C, then freeze drying. After freeze drying, the sample and tube are weighed to calculate the dry matter percentage, then samples are ground to a homogenous powder. The ground, freeze dried meat samples are then placed into a calibrated benchtop NIR spectrophotometer to determine IMF %.

3.4.5 Sensory evaluation

Consumer sensory testing was carried out in line with MSA protocols outlined by Watson et al. (2008). Briefly, all samples were thawed in a fridge (4^0 C for 24 hours) prior to each sensory session before being lifted onto trays 30 mins before cooking to allow them to reach room temperature. A Silex clamshell grill was set at 195°C on the top plate and 210°C on the bottom plate and allowed to heat up 45 minutes prior to cooking the first steaks. Ten starter steaks were cooked and discarded to ensure a stable cooking temperature was achieved. All samples were grilled for 5 minutes and 15 seconds to a medium degree of doneness following the sensory grill protocol. Steaks were placed on the grill in the order they appeared on the sheets to ensure EQSRef codes could be tracked to the consumer.

A common first sample known as a link (LNK) was served to each group of 10 consumers to allow a base for statistical analysis. A 6x6 Latin Square design was employed to ensure each muscle was eaten by the same number of consumers in all serving positions (2-7) effectively balancing the trial for order effects. Each of the 5 samples from each muscle were cut in half into two equal sized rectangular pieces after cooking to allow for consumer testing by 10 consumers. Before calculating the mean sensory scores for each sample, the 10 individual scores for each sample were ranked and the two highest and two lowest scores were clipped to reduce the variance of the mean sensory scores. Each consumer marked a line for tenderness, juiciness, flavour and overall liking on a 100mm line scale for each sample (Watson et al., 2008). In addition, consumers were then asked to mark the sample as unsatisfactory, good everyday, better than everyday or premium quality.

3.4.6 Statistical analysis

Consumer sensory data was processed using MSA modelling technology to create an overall eating quality score (MQ4) for each sample. The MQ4 score is calculated out of 100 using the weighted average of tenderness (0.3), juiciness (0.1), flavour (0.3) and overall liking (0.3) from the 10 consumers per muscle (Bonny et al., 2017).

Data cleaning, data visualisation and models employed the "janitor" (Firke, 2020) , "ggplot2" (Wickham et al., 2016) and "lmerTest" (Kuznetsova et al., 2020) packages respectively using R Core team software (R Core Team, 2022). The effect of muscle, marketing method (treatment group) and their interaction on tenderness, juiciness, flavour, overall liking and MQ4 score were analysed in a linear mixed effect model with animal within producer used as the random term for each replicate. All non-significant terms (P>0.05) were removed from the model. All carcase traits were found to be insignificant except for hump height for Rep 1 and ossification for Rep 2. The combined data was analysed with the treatment and cut plus their interaction as fixed effects and the carcase traits for hot standard carcase weight (HSCW), ossification, marbling and hump were included as covariates. In the combined replicate model, animal within producer and replicate was included as a random term.

4. Results

4.1 Rep 1

4.1.1 Effect of marketing pathway on consumer sensory attributes

The 7 DAY and 14 DAY re-fed treatments had significantly reduced consumer scores across all sensory attributes of MQ4, tenderness, juiciness, flavour and overall liking for both the STR045 and EYE075 ($P < 0.05$) compared to the direct and 72 HR treatment groups, except for juiciness where the 72 HR and 14 DAY treatments did not differ. MQ4 score was lower by 6.40 and 5.94 points for the 7 DAY and 14 DAY re-fed pathways respectively when compared to the directly consigned pathway and this reduction in quality was reflective across all other sensory attributes (Fig. 3, Table 4). The 7 DAY and 14 DAY re-fed treatments did not differ from each other for all sensory attributes of MQ4, tenderness, juiciness, flavour and overall liking for both the STR045 and EYE075 (Fig. 3, $P >$ 0.1). MQ4 and consumer tenderness scores were similar between the directly consigned pathway (59.37 and 51.17 respectively) and the 72 HR pathway (60.17 and 53.66 respectively). The 48-hour treatment also showed no significant difference from the control 24-hour group for MQ4 and tenderness. Although the 48-hour treatment had lower scores than the directly consigned pathway (4.17 and 3.16, respectively), this difference is likely due to random variation rather than a true effect of the treatments. Flavour, juiciness and overall liking score were significantly reduced in the 48 HR treatment compared to the directly consigned and 72 HR pathways ($P < 0.05$).

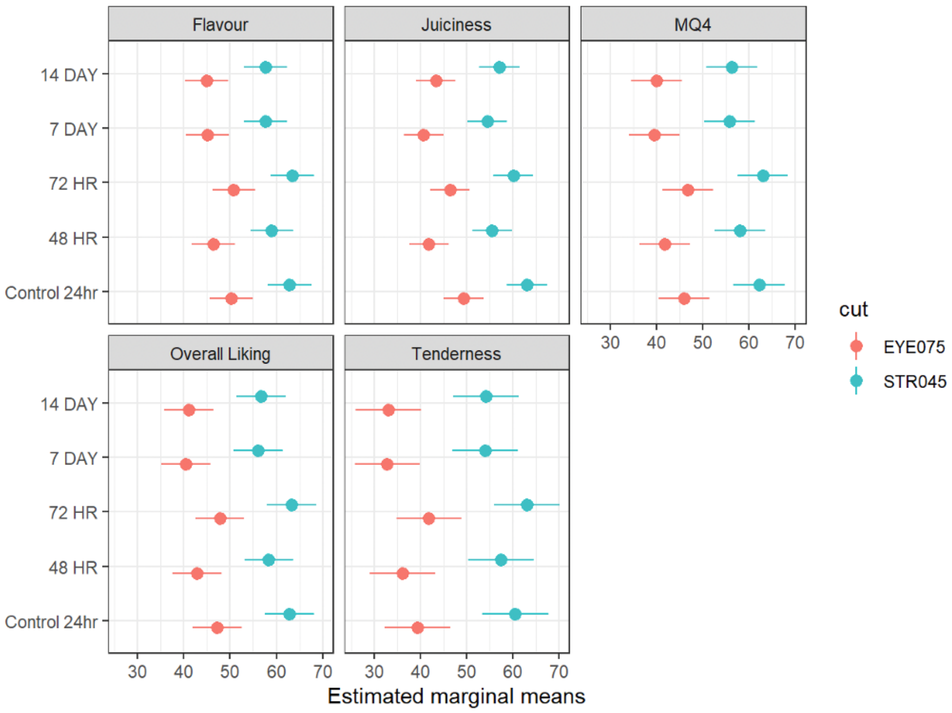

Figure 3. Estimated marginal means including 95% confidence intervals for tenderness, juiciness, flavour, overall liking and MQ4 score for the *M. semitendinosus* (EYE075) and *M. longissimus lumborum* (STR045) for each marketing pathway (Rep 1).

Table 4. Consumer intrinsic attribute scores for MQ4, tenderness, juiciness, flavour and overall liking for the *M. semitendinosus* (EYE075) and *M. longissimus lumborum* (STR045) for each marketing pathway with the inclusion of hump height (Rep 1).

	MQ4		**Tenderness**		**Juiciness**		**Flavour**		**Overall liking**	
Predictors	*Estimates (CI)*	*p*	*Estimates (CI)*	*p*	*Estimates (CI)*	*p*	*Estimates (CI)*	*p*	*Estimates (CI)*	*p*
(Intercept)	59.37 (49.72-69.03)	**<0.001**	51.17 (38.96-63.38)	**<0.001**	65.09 (55.37 – 74.81)	**<0.001**	63.90 (55.32-72.47)	**<0.001**	61.28 (51.59-70.97)	**<0.001**
EYE075	Reference		Reference		Reference		Reference		Reference	
STR045	16.19 (14.18-18.21)	**<0.001**	21.16 (18.70-23.61)	**<0.001**	13.70 (11.64 – 15.77)	**<0.001**	12.64 (10.78-14.50)	**<0.001**	15.57 (13.42-17.73)	**<0.001**
Hump	-0.21 (-0.33- -0.09)	**0.001**	-0.19 (-0.34- -0.04)	**0.015**	-0.25 (-0.38 – -0.13)	**<0.001**	-0.22 (-0.33- -0.11)	**<0.001**	-0.23 (-0.33- -0.10)	**<0.001**
Control 24hr	Reference		Reference		Reference		Reference		Reference	
48 HR	-4.17 (-8.43-0.09)	0.055	-3.16 (- 8.52 – 2.19)	0.246	-7.56 (-12.05 – -3.08)	**0.001**	-3.85 (-7.66 -0.04)	**0.048**	-4.39 (-8.70- -0.09)	**0.045**
72 HR	0.80 (-3.44 -5.04)	0.711	2.49 (-2.85 – 7.83)	0.358	-3.00 (-7.47 – 1.47)	0.187	0.58 (-3.21 – 4.38)	0.763	0.57 (-3.72 -4.85)	0.795
7 DAY	-6.40 (-10.57- -2.23)	**0.003**	-6.53 (-11.78 – -1.28)	**0.015**	-8.66 (-13.05 – -4.26)	**<0.001**	-5.18 (-8.91 - -1.44)	**0.007**	-6.76 (-10.98 - -2.55)	**0.002**
14 DAY	-5.94 (-10.27- -1.61)	**0.007**	-6.31 (-11.77 – -0.86)	**0.023**	-6.02 (-10.59 – -1.46)	**0.010**	-5.28 (-9.16- -1.40)	**0.008**	-6.12 (-10.50 - -1.74)	**0.006**

Control 24hr: Direct consignment, 48 HR: Saleyard 48hr treatment, 72 HR: Saleyard 72hr treatment, 7 DAY: Saleyard 7-day treatment and 14 DAY: Saleyard 14-day treatment. CI – confidence interval.

4.1.2 Effect of muscle on eating quality

Cut had a significant impact on all consumer sensory eating quality attributes (*P* < 0.001). The STR045 muscle consistently had higher scores for all 5 consumer sensory attributes compared to the EYE075 across all treatments. MQ4 score increased by 16.19 between the EYE075 and STR045 (*P* < 0.001) in the directly consigned treatment. The STR045 was 21.16, 13.70, 12.64 and 15.57 points higher than the EYE075 for tenderness, juiciness, flavour and overall liking respectively (Table 4). The interaction between cut and treatment was insignificant across all sensory attributes.

4.1.3 Effect of carcase characteristics on eating quality

Hump height was the only carcase characteristic found to significantly impact consumer sensory eating quality attributes. A 60mm increase in hump height from 40 to 100mm resulted in a 13 point reduction in MQ4 score in both the STR045 and EYE075 muscles (Fig. 4). The reduction in consumer sensory score as hump height increased was also reflected for tenderness, juiciness, flavour and overall liking reflecting the trend observed in MQ4 score. Rib fat, ossification and MSA marble score did not significantly affect consumer intrinsic scores in this group of 120 animals.

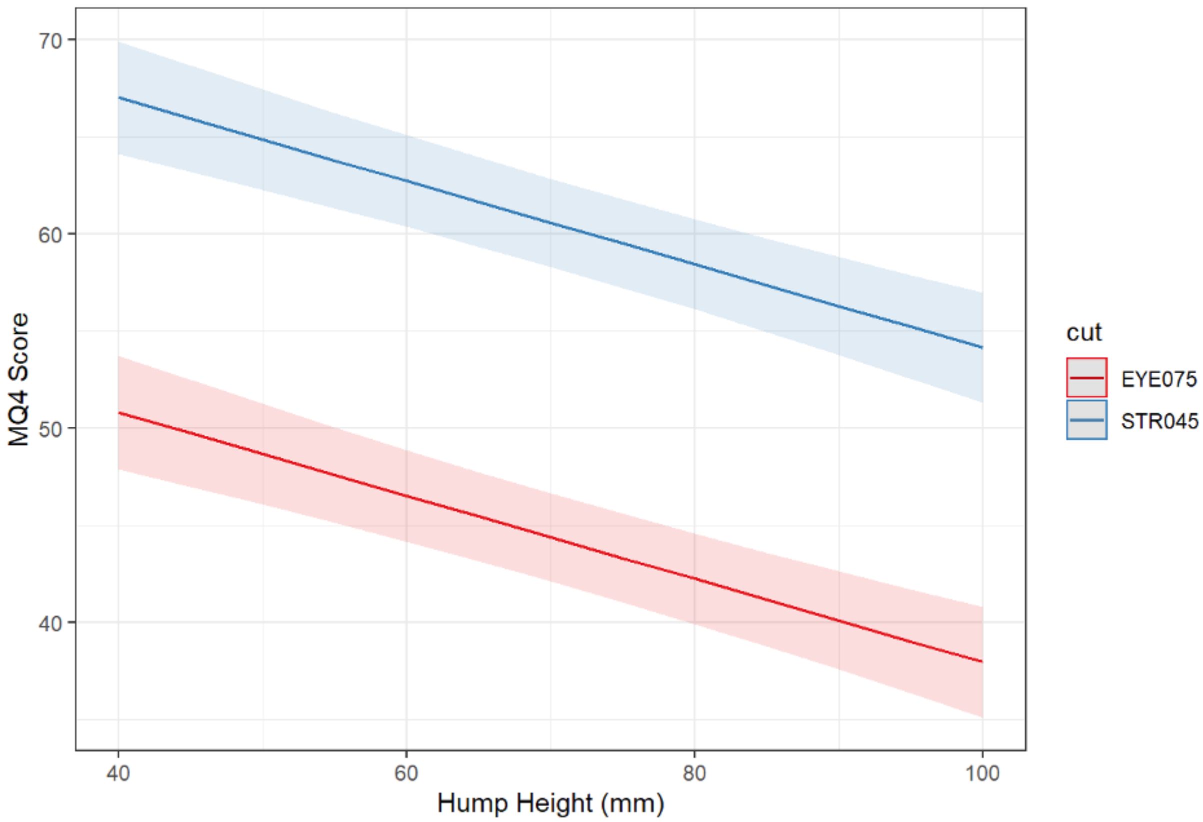

Figure 4. Relationship between consumer MQ4 score and hump height for EYE075 and STR045 displaying 95% confidence interval (Rep 1).

4.1.4 Effect of marketing pathway on objective eating quality measurements

There was no difference in objective eating quality measurements of shear force, IMF % and purge % observed across the 5 treatments (Table 5). The 48hr treatment had a small increase in pH of 0.1

compared to the directly consigned treatment ($P < 0.001$) whilst the 7 DAY treatment observed a 0.06 unit reduction in pH ($P = 0.006$). The 7 DAY and 14 DAY treatments had a slightly higher cook loss percentage (23.61 and 23.9%, respectively) compared to the control (22.42%, $P < 0.01$).

Table 5. Objective eating quality measurements for shear force, IMF %, pH, purge % and cook loss % per treatment (Rep 1).

	Shear Force		**IMF %**		**pH**		**Purge %**		**Cook Loss %**	
Predictors	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*
Control 24hr (Intercept)	5.51	**<0.001**	2.49	**<0.001**	5.66	**<0.001**	7.98	**<0.001**	22.42	**<0.001**
48 HR	-0.13	0.476	0.21	0.431	0.10	**<0.001**	0.57	0.657	-0.01	0.983
72 HR	-0.28	0.129	0.15	0.579	0.01	0.641	2.44	0.056	-0.18	0.685
7 DAY	0.26	0.148	0.31	0.246	-0.06	**0.006**	2.39	0.061	1.19	**0.007**
14 DAY	-0.16	0.395	0.20	0.458	-0.02	0.239	1.59	0.214	1.48	**0.001**

Control 24hr: Direct consignment, 48 HR: Saleyard 48hr treatment, 72 HR: Saleyard 72hr treatment, 7 DAY: Saleyard 7-day treatment and 14 DAY: Saleyard 14-day treatment

4.1.5 Effect of marketing pathway on weight and dressing percentage

Marketing pathway had a significant impact on the start weight in 48 HR with a reduction of 23.21kg (P <0.05) and finish liveweight of animals in both the 48 HR and 72 HR pathways with reductions of 52.13 and 34.46kg respectively (P <0.05) compared to the directly consigned control group. The 7 DAY and 14 DAY re-fed treatment had weights similar to the directly consigned pathway (Table 6). No significant differences in start weight or carcase weight were evident between any of the 5 treatments.

Table 6. Weight measurements for start weight (farm exit weight), finish weight, carcase weight and dressing percentage per treatment (Rep 1) from start weight and finish weight (weight at saleyard exit).

	Start Weight		**Finish Weight**		**Carcase Weight**		**Dressing % (from start weight)**	**Dressing % (from finish weight)**
Predictors	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*		
(Intercept)	537.05	**<0.001**	537.05	**<0.001**	280.43	**<0.001**	52.74	NA
Control 24hr	*Reference*		*Reference*		*Reference*			
48 HR	-23.21	**0.028**	-52.13	**<0.001**	-14.14	0.025	51.81	54.87
72 HR	-12.42	0.409	-34.46	**0.001**	-7.54	0.400	52.09	54.52
7 DAY	-9.78	0.519	-19.13	0.212	-7.35	0.412	52.02	52.72
14 DAY	-4.13	0.783	-0.05	0.998	0.55	0.951	52.75	52.45

Control 24hr: Direct consignment, 48 HR: Saleyard 48hr treatment, 72 HR: Saleyard 72hr treatment, 7 DAY: Saleyard 7-day treatment and 14 DAY: Saleyard 14-day treatment

4.1.6 Effect of marketing pathway on carcase characteristics

Hump height in the direct consignment treatment was significantly higher than all other treatments by between 12.92 and 8.33mm ($P < 0.05$, Table 7). Rib fat and ossification were significantly different by -1.67 and 0.045 for the 72 HR and 48 HR, respectively from the Control 24hr group.

Table 7: Carcase attribute measurements for eye muscle area, rib fat, P8 fat, hump height, ossification and marbling per treatment (Rep 1).

	Eye Muscle Area		Rib Fat		P8 Fat		Hump Height		Ossification		Marbling	
Predictors	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*
(Intercept)	77.00	**<0.001**	6.92	**<0.001**	10.12	**<0.001**	71.04	**<0.001**	170.00	**<0.001**	320.00	**<0.001**
Control 24hr	Reference		Reference		Reference		Reference		Reference		Reference	
48 HR	-2.21	0.247	-0.38	0.556	-0.62	0.424	-11.04	**<0.001**	-15.83	**0.045**	-17.50	0.186
72 HR	-0.88	0.646	-1.67	**0.009**	-1.33	0.089	-10.63	**<0.001**	-15.42	0.050	-7.08	0.592
7 DAY	0.92	0.631	-1.17	0.068	-1.92	**0.015**	-8.33	**<0.001**	-5.42	0.490	0.42	0.490
14 DAY	-1.83	0.337	-0.54	0.396	-0.87	0.263	-12.92	**<0.001**	-7.50	0.340	-19.17	0.340

Control 24hr: Direct consignment, 48 HR: Saleyard 48hr treatment, 72 HR: Saleyard 72hr treatment, 7 DAY: Saleyard 7-day treatment and 14 DAY: Saleyard 14-day treatment

4.1.7 Effect of marketing pathway on incidence of dark cutting

Fig. 5 shows the percentage of carcases with a pH_u > 5.7 and those with an AUS-MEAT colour > 3. The directly consigned treatment had 20.8% of carcases with a pH_u > 5.7, being 4.2% higher than both the 48 HR and 7 DAY treatment and 8.3% higher than the 72 HR and 14 DAY treatments. The 72 HR treatment had the highest proportion of carcases with an AUS-MEAT colour greater than 3, with all treatments having a greater number of carcases with an AUS-MEAT colour > 3 than a pH_u > 5.7. The 14 DAY re-fed treatment had the equally lowest proportion of carcases with a pH_u > 5.7 (12.5%) and AUS-MEAT colour > 3 (50%). The meat colour scores could have contained some grader bias as the graders were aware of the project methodology.

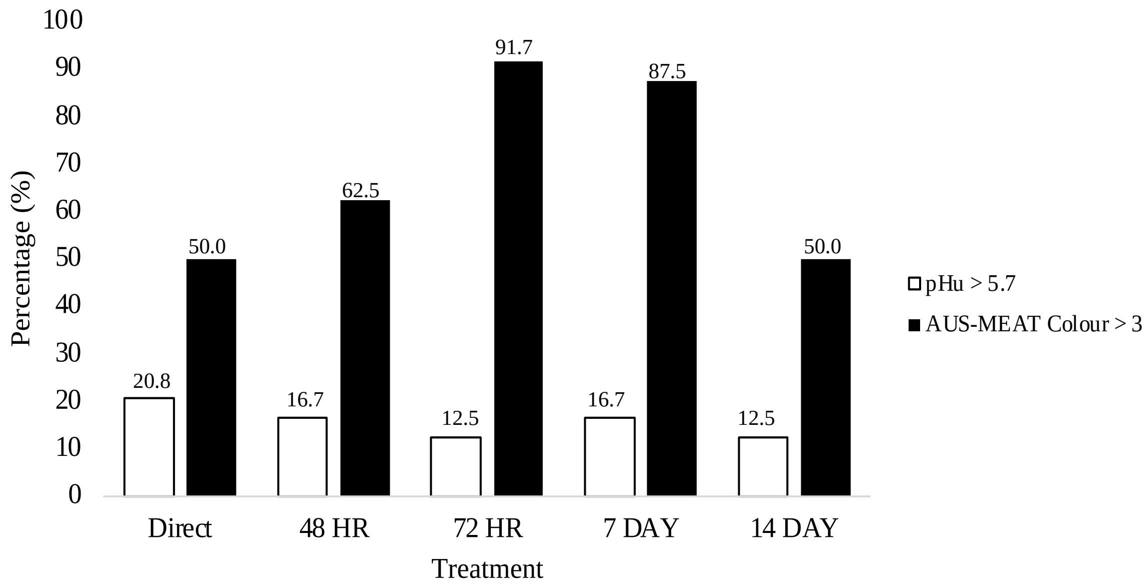

Figure 5. Percentage of animals per treatment group with a pHu > 5.7 and AUS-MEAT colour > 3

4.2 Rep 2

4.2.1 Effect of marketing pathway on consumer sensory attributes

The 14 DAY re-fed treatment had significantly improved consumer scores by 3.63 in flavour for both the STR045 and EYE075 ($P < 0.05$) compared to the direct consignment (Control 24hr) group (Fig. 6, Table 8), while there was no significant difference on flavour across other treatment groups. The 48 HR (water only) and any other re-fed treatment groups had no impact on all other sensory attributes of MQ4, tenderness, juiciness and overall liking ($P > 0.05$). Although all consumer sensory attributes for MQ4, tenderness, juiciness, flavour and overall liking were improved across all treatment groups, none of the differences were statistically significant.

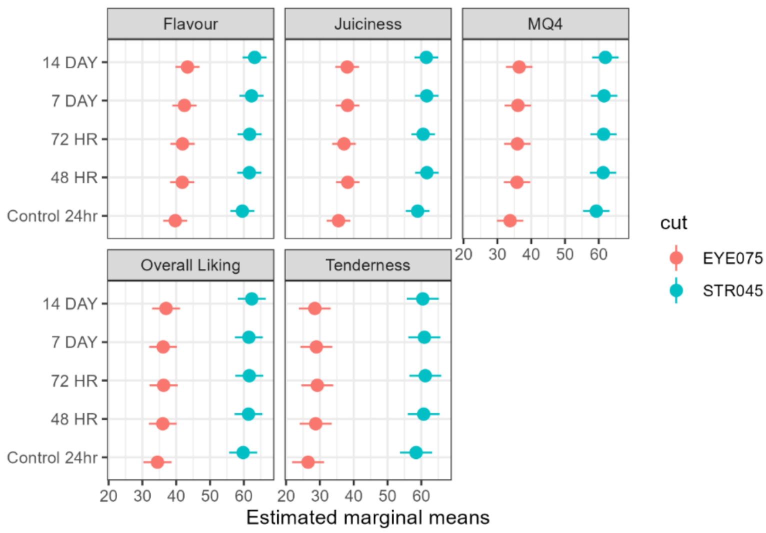

Figure 6. Estimated marginal means including 95% confidence intervals for tenderness, juiciness, flavour, overall liking and MQ4 score for the M. semitendinosus (EYE075) and M. longissimus lumborum (STR045) for each marketing pathway (Rep 2)

Table 8. Consumer intrinsic attribute scores for MQ4, tenderness, juiciness, flavour and overall liking for the *M. semitendinosus* (EYE075) and *M. longissimus lumborum* (STR045) for each marketing pathway with the inclusion of ossification (Rep 2)

	MQ4		**Tenderness**		**Juiciness**		**Flavour**		**Overall liking**	
Predictors	*Estimates (CI)*	*p*	*Estimates (CI)*	*p*	*Estimates (CI)*	*p*	*Estimates (CI)*	*p*	*Estimates (CI)*	*p*
(Intercept)	40.79 (31.09-50.50)	**<0.001**	39.98 (28.08-51.89)	**<0.001**	34.97 (25.73-44.20)	**<0.001**	43.47 (34.20-52.74)	**<0.001**	40.87 (30.75-50.99)	**<0.001**
EYE075	Reference		Reference		Reference		Reference		Reference	
STR045	25.49 (23.35-27.64)	**<0.001**	31.96 (**29.36-34.56)**	**<0.001**	23.42 (21.13-25.70)	**<0.001**	19.86 (17.67-22.04)	**<0.001**	25.36 (23.14-27.59)	**<0.001**
Control 24hr	Reference		Reference		Reference		Reference		Reference	
48 HR	2.03 (-1.52-5.59)	0.260	2.23 (-2.09-6.56)	0.310	2.75 (-1.02-6.51)	0.152	2.06 (-1.54-5.67)	0.261	1.57 (-2.11-5.25)	0.401
72 HR	2.15 (-1.60-5.89)	0.260	2.71 (-1.86-7.29)	0.244	1.64 (-2.31-5.59)	0.415	2.16 (-1.63-5.95)	0.264	1.81 (-2.06-5.68)	0.358
7 DAY	2.30 (-1.42-6.03)	0.225	2.45 (-2.12-7.01)	0.292	2.70 (-1.24-6.64)	0.178	2.71 (-1.06-6.49)	0.158	1.68 (-2.18-5.54)	0.392
14 DAY	2.68 (-0.86-6.23)	0.137	1.98 (-2.34-6.30)	0.367	2.58 (-1.18-6.34)	0.178	3.63 (0.03-7.23)	**0.048**	2.54 (-1.13-6.22)	0.174
Ossification	-0.04 (-0.09 – 0.01)	0.136	-0.08 (-0.14- -0.01)	**0.021**	0.00 (-0.05-0.05)	0.909	-0.02 (-0.07-0.03)	0.404	-0.04 (-0.09-0.02)	0.191

Control 24hr: Direct consignment, 48 HR: Saleyard 48hr treatment, 72 HR: Saleyard 72hr treatment, 7 DAY: Saleyard 7-day treatment and 14 DAY: Saleyard 14-day treatment CI – confidence interval.

4.2.2 Effect of muscle on eating quality

Cut had a significant impact on all consumer sensory eating quality attributes ($P < 0.001$, Table 8). The STR045 muscle consistently had higher scores for all 5 consumer sensory attributes compared to the EYE075 across all treatments. The STR045 was 25.49, 31.96, 23.42, 19.86, and 25.36 points higher than the EYE075 for MQ4, tenderness, juiciness, flavour and overall liking respectively (Table 8). The interaction between cut and treatment was insignificant across all sensory attributes.

4.2.3 Effect of carcase characteristics on eating quality

Ossification was the only carcase characteristic found to significantly impact consumer sensory eating quality attributes for tenderness ($P < 0.001$, Table 8). An increase in ossification from 100 to 250 resulted in a 10-point reduction in tenderness score in both the STR045 and EYE075 muscles (Fig. 7). Hump height, P8 fat, eye muscle area, rib fat, pHu and MSA marble score did not significantly affect consumer intrinsic scores in this replication.

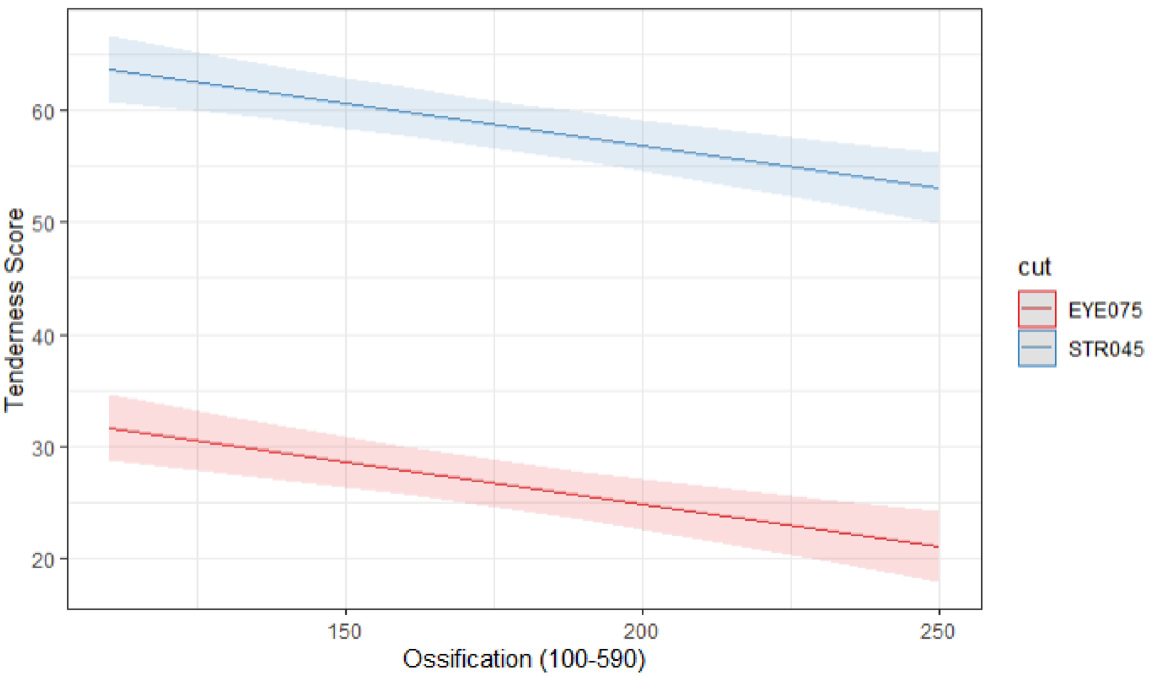

Figure 7. Relationship between consumer tenderness score and ossification for EYE075 and STR045 displaying 95% confidence interval (Rep 2)

4.2.4 Effect of marketing pathway on weight and dressing percentage

No significant differences in start weight, finish weight, carcase weight and dressing percentage related to start weight and finish weight were found between any of the marketing pathways (Table 9). The finish weight was improved for 48 HR group and all treatment groups had lower weight traits compared to control 24hr group, although none of these results were statistically significant (P >0.05).

Table 9: Weight measurements for start weight (farm exit weight), finish weight, carcase weight and dressing percentage per treatment (Rep 2) from start weight and finish weight (weight at saleyard exit).

	Start Weight		Finish Weight		Carcase Weight		Dressing % (from start weight)	Dressing % (from finish weight)
Predictors	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*		
(Intercept)	580.04	**<0.001**	577.04	**<0.001**	294.41	**<0.001**		
Control 24hr	Reference		Reference		Reference		50.76	NA
48 HR	-0.94	0.942	0.71	0.956	-0.29	0.967	50.78	50.91
72 HR	-16.19	0.211	-15.06	0.237	-7.13	0.307	50.95	51.11
7 DAY	-18.85	0.145	-21.06	0.098	-8.51	0.223	50.93	51.40
14 DAY	-13.10	0.311	-14.58	0.252	-5.56	0.425	50.94	51.35

Control 24hr: Direct consignment, 48 HR: Saleyard 48hr treatment, 72 HR: Saleyard 72hr treatment, 7 DAY: Saleyard 7-day treatment and 14 DAY: Saleyard 14-day treatment

4.2.5 Effect of marketing pathway on carcase characteristics

There was no significant difference in any of the recorded carcase characteristics across the 5 treatment groups except for ossification (Table 10). Ossification score was increased by 14.4 points in the 72 HR treatment group compared to the direct consignment group ($P < 0.05$).

Table 10. Carcase attribute measurements for farm weight, carcase weight, eye muscle area, rib fat, p8 fat, hump height, ossification, marbling per treatment (Rep 2).

	Eye Muscle Area		Rib Fat		P8 Fat		Hump Height		Ossification		Marbling	
Predictors	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*
(Intercept)	76.31	**<0.001**	5.81	**<0.001**	12.75	**<0.001**	60.83	**<0.001**	172.29	**<0.001**	345.83	**<0.001**
Control 24hr	Reference		Reference		Reference		Reference		Reference		Reference	
48 HR	0.73	0.664	0.04	0.929	0.17	0.868	-0.62	0.778	5.83	0.378	11.25	0.542
72 HR	1.54	0.359	0.06	0.894	0.52	0.603	-3.85	0.084	14.38	**0.031**	6.25	0.735
7 DAY	-0.23	0.891	0.02	0.964	0.52	0.603	-3.33	0.134	11.04	0.096	12.29	0.5056
14 DAY	0.10	0.951	0.31	0.504	0.38	0.708	-1.35	0.542	2.29	0.729	7.71	0.676

Control 24hr: Direct consignment, 48 HR: Saleyard 48hr treatment, 72 HR: Saleyard 72hr treatment, 7 DAY: Saleyard 7-day treatment and 14 DAY: Saleyard 14-day treatment

4.2.6 Effect of marketing pathway on incidence of dark cutting

Fig. 8 shows the percentage of carcases with a $pH_u > 5.7$ and those with an AUS-MEAT colour > 3. The directly consigned treatment and 48 HR had 10.8% of carcases with a $pH_u > 5.7$, being 1.6% higher than both the 72 HR and 7 DAY treatment and 2.2% lower than the 14 DAY treatments. The 48 HR and 72 HR treatment groups had the highest proportion of carcases with an AUS-MEAT colour greater than 3, with all treatments having a moderate number of carcases with an AUS-MEAT colour > 3 than a $pH_u > 5.7$ (Fig. 8). The 7 DAY re-fed treatment had the equally lowest proportion of carcases with a $pH_u > 5.7$ (9.2%) and lowest AUS-MEAT colour > 3 (26.7%).

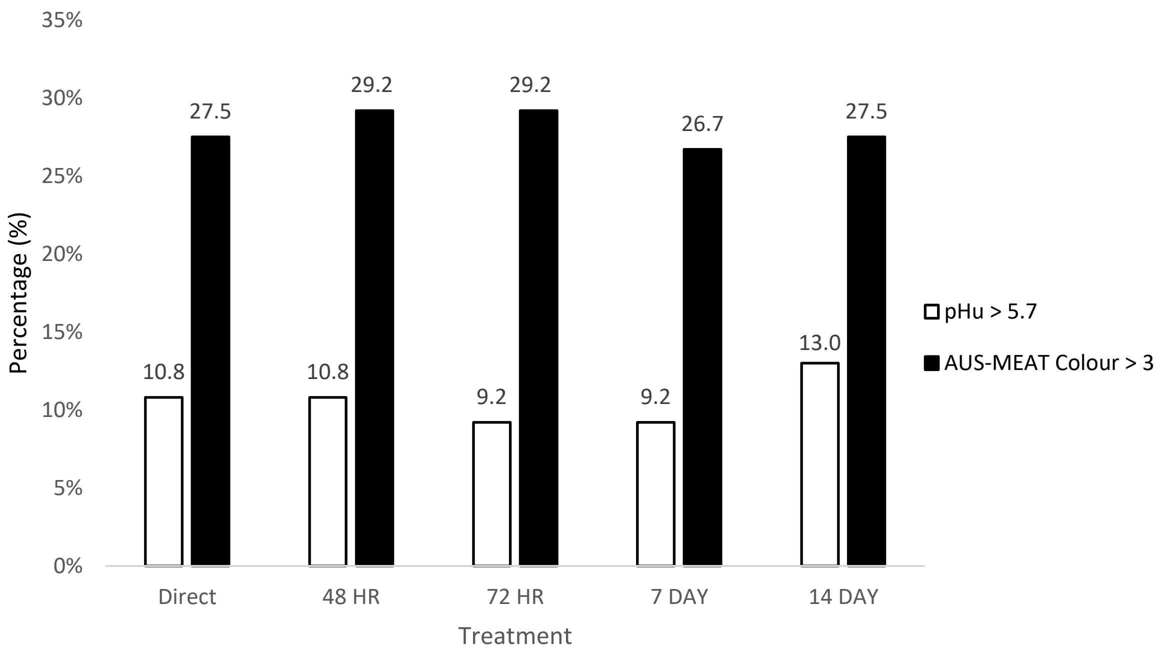

Figure 8. Percentage of animals per treatment group with a $pH_u > 5.7$ and AUS-MEAT colour > 3 (Rep 2)

4.3 Combined analysis (Rep 1 and Rep 2)

4.3.1 Effect of marketing pathway on consumer sensory attributes

The 72 HR re-fed treatment had numerically improved consumer scores across all sensory attributes of MQ4, tenderness, juiciness, flavour and overall liking for both the STR045 and EYE075 compared to the other treatment groups (Table 11), although it was not significant. The 48 HR, 7 DAY and 14 DAY re-fed treatment had reduced consumer score across all sensory attributes compared to the direct consignment group, however again this was not significant. MQ4 score was lower by 0.13, 1.32 and 0.47 points for the 48 HR, 7 DAY and 14 DAY re-fed pathways respectively when compared to the directly consigned pathway and this reduction in quality was reflective across all other sensory attributes. Overall, all treatment group had a minor numerical impact (0-2 points) on consumer sensory attributes when both replicates combined, although none of the differences were statistically significant.

Table 11. Consumer intrinsic attribute scores for MQ4, tenderness, juiciness, flavour and overall liking for the *M. semitendinosus* (EYE075) and *M. longissimus thoracis et lumborum* (STR045) for each marketing pathway with the inclusion of ossification and MSA Marbling for combined analysis (Rep 1 and Rep 2).

	MQ4		**Tenderness**		**Juiciness**		**Flavour**		**Overall liking**	
Predictors	*Estimates (CI)*	*p*	*Estimates (CI)*	*p*	*Estimates (CI)*	*p*	*Estimates (CI)*	*p*	*Estimates (CI)*	*p*
(Intercept)	44.40(34.58-54.23)	**<0.001**	43.88 (31.92 – 55.85)	**<0.001**	38.54 (28.44 – 48.63)	**<0.001**	47.33 (38.05 – 56.60)	**<0.001**	46.15 (35.18-57.13)	**<0.001**
STR045	20.84 (19.25-22.44)	**<0.001**	26.56 (24.64 – 28.47)	**<0.001**	18.56 (16.90 – 20.22)	**<0.001**	16.25 (14.74-17.76)	**<0.001**	21.69 (19.83-23.55)	**<0.001**
Hump	-0.08 (-0.17-0.01)	0.078	-0.08 (-0.19-0.03)	0.108	-0.05 (-0.16-0.05)	0.299	-0.07 (-0.16-0.01)	0.061	-0.08 (-0.18-0.02)	0.115
Control 24hr	Reference		Reference		Reference		Reference		Reference	
48 HR	-0.13 (-2.94-2.68)	0.609	0.12 (-3.31 – 3.55)	0.737	-0.72 (-3.66 – 2.22)	0.395	0.18 (-2.48-2.86)	0.760	-0.89 (-4.08-2.29)	0.489
72 HR	1.82 (-1.06-4.70)	0.255	2.48 (-1.05 – 6.02)	0.213	0.37 (-2.65 – 3.38)	0.821	1.90(-0.84-4.65)	0.204	1.56 (-1.69-4.80)	0.340
7 DAY	-1.32 (-4.18-1.54)	0.252	-1.43 (-4.94 – 2.08)	0.275	-1.76 (-4.76 – 1.23)	0.161	-0.47 (-3.20-2.25)	0.556	-1.91(-5.13-1.30)	0.161
14 DAY	-0.47 (-3.29-2.35)	0.492	-1.27 (-4.71-2.18)	0.265	-0.04 (-2.99-2.92)	0.800	0.46 (-2.23-3.15)	0.950	-0.83 (-4.04-2.37)	0.468
Marbling	0.02 (0.00-0.03)	**0.003**	0.02 (-0.00 – 0.03)	0.05	0.02 (0.01 -0.04)	**<0.001**	0.02 (0.00-0.03)	**0.006**	0.02 (0.00-0.03)	**0.042**
Ossification	-0.03 (-0.07-0.00)	0.068	-0.07 (-0.11- -0.03)	**<0.001**	-0.01 (-0.05-0.03)	0.075	-0.02 (-0.05-0.02)	0.284	-0.06 (-0.08-0.00)	0.137

Control 24hr: Direct consignment, 48 HR: Saleyard 48hr treatment, 72 HR: Saleyard 72hr treatment, 7 DAY: Saleyard 7-day treatment and 14 DAY: Saleyard 14-day treatment. CI – confidence interval.

4.3.2 Effect of carcase characteristics on eating quality

Marbling had significant effect on MQ4, juiciness, flavour and overall liking for both the STR045 and EYE075 cuts (*P* < 0.05, Table 12). Marbling significantly improved the MQ4, juiciness, flavour and overall liking score each by 0.02. MQ4 score points for every 1 point increase in MSA Marbling. Ossification significantly reduced the MQ4, tenderness and overall likeness by 0.06, 0.09 and 0.06 score points, respectively compared to the control group for every 1 point increase in ossification.

4.3.3 Effect of marketing pathway on weight and dressing percentage

Marketing pathway had a significant impact on the finish liveweight of animals in the 48 HR, 72 HR and 7 DAY pathways with reductions of 26.58, 25.63 and 20.97 kg respectively (*P* <0.05) compared to the directly consigned control group. The 14 DAY re-fed treatment had weight similar to the directly consigned pathway (Table 12). No significant differences in start weight or carcase weight were evident between any of the 5 treatments.

Table 12: Weight measurements for start weight (farm exit weight), finish weight, carcase weight and dressing percentage per treatment (Rep 1 and Rep 2) from start weight and finish weight (weight at saleyard exit).

	Start Weight		Finish Weight		Carcase Weight		Dressing % (from start weight)	Dressing % (from finish weight)
Predictors	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*		
(Intercept)	559.48	**<0.001**	557.91	**<0.001**	287.42	**<0.001**		
STR045	-0.00	1.000	-0.00	1.000	0.00	1.000		
Control 24hr	Reference		Reference		Reference		52.74	NA
48 HR	-13.01	0.145	-26.58	**0.004**	-7.22	0.134	51.81	54.87
72 HR	-15.24	0.088	-25.63	**0.006**	-7.33	0.128	52.19	54.52
7 DAY	-14.89	0.097	-20.97	**0.024**	-7.93	0.100	52.02	52.72
14 DAY	-9.55	0.285	-8.18	0.396	-2.51	0.602	52.75	52.45

Control 24hr: Direct consignment, 48 HR: Saleyard 48hr treatment, 72 HR: Saleyard 72hr treatment, 7 DAY: Saleyard 7-day treatment and 14 DAY: Saleyard 14-day treatment

4.3.4 Effect of marketing pathway on carcase characteristics (Rep 1 and Rep 2)

Only hump height in the direct consignment treatment was significantly higher than all other treatments by between 5.83 and 7.24mm (*P* < 0.05, Table 13).

Table 13: Carcase attribute measurements for eye muscle area, rib fat, P8 fat, hump height, ossification and marbling per treatment (Rep 1 and Rep 2).

	Eye Muscle Area		Rib Fat		P8 Fat		Hump Height		Ossification		Marbling	
Predictors	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*
(Intercept)	76.66	**<0.001**	6.36	**<0.001**	11.44	**<0.001**	65.94	**<0.001**	171.15	**<0.001**	332.92	**<0.001**
Control 24hr	Reference		Reference		Reference		Reference		Reference		Reference	
48 HR	-0.74	0.559	-0.17	0.673	-0.23	0.741	-5.83	**<0.001**	-5.00	0.347	-3.12	0.789
72 HR	0.33	0.792	-0.80	0.043	-0.41	0.558	-7.24	**<0.001**	-0.52	0.922	-0.42	0.972
7 DAY	0.34	0.786	-0.57	0.148	-0.70	0.314	-5.83	**<0.001**	2.81	0.596	6.35	0.587
14 DAY	-0.86	0.495	-0.11	0.772	-0.25	0.718	-7.14	**<0.001**	-2.60	0.624	-5.73	0.624

Control 24hr: Direct consignment, 48 HR: Saleyard 48hr treatment, 72 HR: Saleyard 72hr treatment, 7 DAY: Saleyard 7-day treatment and 14 DAY: Saleyard 14-day treatment, 72 HR: Saleyard 72hr treatment, 7 DAY: Saleyard 7-day treatment and 14 DAY: Saleyard 14-day treatment

5. Discussion

5.1 Intrinsic eating quality

Historically, additional preslaughter stressors associated with the saleyard pathway have adversely impacted carcase eating quality attributes, however, the extent of the effect has been highly variable between trials (Ferguson et al., 2007b, Shorthose, 1988). In the present study, a reduction in consumer MQ4 score of 4.17 were observed in the 48 HR compared to cattle directly consigned (P = 0.055) for Rep 1, whereas no significant effect of treatments had been found across all meat quality traits of Rep 2 and combined data. This reduction in consumer MQ4 score in Rep 1 aligns well and supports the current 5 point penalty applied by MSA to cattle marketed through the saleyard pathway (Watson et al., 2008). These results are contrary to studies by Loudon et al. (2019) and Ferguson et al. (2007b) that found limited to no significant difference in consumer MQ4 score between the saleyard and direct consignment pathways. These results, however, aligned with the Rep 2 and combined results. Although both Ferguson et al. (2007b) and Warner et al. (1998) found that cattle marketed through saleyards tended to be tougher, however, the small statistical power of these studies resulted in no significant differences, implying marketing method has a relatively small but variable effect on the tenderness of beef. The size of effect is surprisingly small given the compelling amount of literature finding adverse eating quality impacts of individual stressors that animals experience under saleyard conditions such as mixing, fasting and increased lairage time (Colditz et al., 2007, Warriss, 1990, Ferguson and Warner, 2008, Eldridge et al., 1984).

5.2 Impact of re-feeding on beef eating quality

The impact of re-feeding on beef eating quality contradicted our hypothesis as our results showed a reduction of greater than 5 points across all consumer sensory traits in the 7 DAY and 14 DAY re-fed treatments compared to cattle that were directly consigned in Rep 1. However, no significant effects on eating quality were found in Rep 2 other than improved flavour score in 14 DAY treatment beef. Previous literature has demonstrated a positive interaction between re-feeding and eating quality, however, this has been related to the positive effect re-feeding had on muscle glycogen and subsequent reduction in dark cutting meat (McVeigh and Tarrant, 1982, Shorthose et al., 1972, Leo-Penu et al., 2015).

The adverse impact observed in the 7 DAY treatment in Rep 1 could be explained by the presence of chronic stressors due to inadequate time to establish social hierarchy after contemporary group mixing, with a minimum of 3 to 4 days required (Doyle and Moran, 2015). The reduced tenderness and MQ4 score of the 7 DAY pathway may also be attributed to decreased proteolysis caused by insufficient nutritional gain in the period prior to slaughter. Proteolytic activity is known to be induced by nutritional means in vivo and thus may be related to the extent of proteolysis post-mortem and myofibril fragmentation that has a direct link to beef tenderness (Boisclair et al., 1993, Vestergaard et al., 2000). This adverse impact was also observed in the 14 DAY treatment in Rep 1 indicated by a 6.31 point reduction in consumer tenderness score suggesting a sustained stress impact on cattle throughout the re-feeding process. This finding has not been recorded in previous literature with cattle rested for 2 weeks on pasture at the processing plant post saleyard exposure having improved eating quality scores compared to the current study (Loudon et al., 2019). Thus, it is hypothesised that cattle re-fed at the saleyard in the current trial were exposed to chronic stressors throughout the re-feeding process including the arrival and dispatch of multi-vendor cattle at the facility as well as human and machine movements within proximity resulting in a sustained stress effect. Wythes et al. (1988) demonstrated adverse tenderness impacts of disruptions in the resting period of cattle preslaughter, recording a 0.17 to 1.08kg increase in shear force compared to cattle with no disruptions. Surprisingly, this adverse impact was not observed in the 72 HR treatment for any consumer sensory trait or MQ4 score in Rep 1 and in combined trial, implying prolonged exposure to stress has greater impact on eating quality than an initial stress event. Overall, the effect of re-feeding was inconsistent across replicates and combined data. Future research into the level of stress experienced during the re-feeding period through the utilisation of biological markers could be utilised in additional replicates in order to understand the animals biological stress. state across the trial. Additionally, the results possibly indicate that the environmental variation, feeding management, and the social interactions between cattle have a bigger effect on eating quality outcome than the feed offered.

It was hypothesised that sustained or chronic exposure to a stressful environment at the saleyard resulted in the 7 DAY and 14 DAY re-fed treatments eating quality 7.20 and 6.47 points respectively lower than the 72 HR treatment for Rep 1, despite the same environment. This hypothesis aligns with findings from Loudon et al. (2019) who concluded duration of preslaughter stress exposure impacts MQ4 score. The improvement in eating quality between the 48 HR treatment and the 72 HR treatment in Rep 1 was unexpected given the additional stress of mixing contemporary groups in the 72 HR re-fed pathway. Although MQ4 and tenderness were not significantly different between the treatments, the 48 HR treatment reflective of an extension of the current MSA pathway (36 hours + 12 hours with water only), had lower values for all intrinsic eating quality attributes for both replicates than the controls. This result suggests that nutritional stress associated with 48 HR treatment group could have a negative effect on eating quality (juiciness, flavour and overall liking) than mixing of contemporary groups with access to feed in this trial, which could be adjusted with a 5 point penalty applied to the predictions of cut x cook combinations. On the contrary, Rep 2 and combined trial did not show any significant difference between control and extended treatment groups that could be because of fundamental differences between the properties, environment, feed, breed and saleyard management which are all confounded within the property. Therefore, these results suggest that the current MSA pathway could be extended to 48 hours with the 5 point penalty applied to overcome the small negative numerical effect on eating quality attributes, particularly on MQ4 and tenderness. Given the variability in previous literature regarding the impact of mixing on eating quality (Colditz et al., 2007, Warriss, 1990, Jones and Tong, 1989) it is plausible the mixing of vendor groups and other stressors as part of the saleyard pathway in this study had little effect on eating quality. Further replication of the trial would be required to affirm this hypothesis and to determine if a short re-feed period can minimise the eating quality impact of cattle exposed to saleyards.

An alternative theory is that the eating quality of the control group was also affected due to the establishment of the control groups (6 head from each farm) on the day of trucking to the processing plant. These groups, even though they were contemporary's in a larger mob were also drafted into a new group, suggesting that social hierarchy would have also been re-established during trucking and lairage in the control group. As suggested above this may have caused stress within the control group and had an unmeasurable adverse effect on the eating quality of beef from the control group. An alternative methodology discussed at length for this project was to draft off the control cattle 2 weeks prior to consignment but they would have needed to be in a different paddock potentially adding a much larger nutrition effect to the meat quality outcomes of the project.

In addition, the insignificant interaction between treatment and cut meant the same effect was observed in both the EYE075 and STR045 for both replicates in this experiment. This was unexpected given the difference in muscle type and workload between the 2 muscles. Previous work by Loudon et al. (2019) found stress associated with mixing cattle significantly reduced the eating quality of the outside, with unexpected variability observed in the eating quality of the eye round. Meat quality scores for the striploin and tenderloin were not significantly different across any of the treatment groups and trials as expected given their muscle type and position (Loudon et al. 2019).

5.3 Objective eating quality for Rep 1

Treatment had an insignificant effect on shear force in Rep 1 meaning the reduced consumer tenderness scores observed in the 7 DAY and 14 DAY re-fed treatment is likely the result of factors influencing consumer mastication or other traits. Reduced juiciness in both the 7 DAY and 14 DAY re-fed treatments may have influenced the lower consumer scores for the other traits, as they are highly correlated ($r > 0.848$). Preslaughter stress has an adverse impact on meat water holding capacity and thus juiciness, through both independent and pH interactions (Van der Wal et al., 1999, Warner et al., 2007). The reduced juiciness consumer sensory scores due to inferior water holding capacity induced by stress supports the previous hypothesis that the 7 DAY and 14 DAY re-fed treatments suffered sustained stress. Warner et al. (2007) previously found that cattle subjected to acute preslaughter stress had a 4 point reduction in consumer MQ4 scores, however, shear force at both 2 and 21 days did not differ between treatments. Inferior water holding capacity can also negatively impact cook loss as they are inversely related (Warner, 2017), which is a possible mechanism why a 1.19% and 1.48% increase in cook loss was observed in the 7 day and 14 day re-fed treatments, respectively, in this study. Purge percentage was not statistically different across any of the pathways in Rep 1, however treatments exposed to saleyards had slightly higher purge percentages of between 0.57% and 2.44% when compared to the direct 24hr treatment, even though these were not statistically significant. In contrast to predictions, Ijaz et al. (2020) identified a link between increased purge and cook loss in cases of decreased water holding capacity. Furthermore, a 1.9% rise in the purge percentage was noted by (Warner et al., 2007) in cattle that were under acute stress. Both the purge percentage of cattle acutely stressed with electric stimulation prior to slaughter and the control treatment were much lower than the current study despite a longer ageing period of 21 days (Warner et al., 2007). There was no relationship between treatment, re-feed time and mixing exposure on intramuscular fat or marbling which aligns with previous literature (Clariget et al., 2021, Carr et al., 1971).

5.4 Liveweight and yield

The 48 HR saleyard pathway and 72 HR short re-fed period adversely affected the final liveweight of cattle by 52.13 and 34.46kg respectively ($p < 0.05$) for Rep 1; however, Rep 2 presented numerically lower liveweight and carcase weights for every treatment group as compared to the direct 24-hour treatment, but no statistically significant change in these weights was observed. In the combined trial, the final live weight of cattle was decreased by 26.58, 25.63 and 20.97kg for the 48 Hr, 72 HR and 7 DAY treatments, respectively. The final liveweight loss of the short saleyard treatments largely reflect reduced gut fill associated with excretion of urine and faeces accounting for 12 to 22% of the liveweight of cattle (Tayler, 1954). Furthermore, the 48 HR and 72 HR pathways had the lowest on farm (start) liveweights of all the treatment groups being 23.21 ($p = 0.124$) and 12.42kg ($p = 0.409$) lower than the directly consigned group in Rep 1, however, this difference was not statistically significant. Shorthose and Wythes (1988) determined the greatest rate of liveweight loss occurs in the first 12 hours of fasting, with carcase weight loss not typically observed until greater than 24 to 48 hours off feed, at which point losses can be attributed to tissue catabolism and dehydration. As a result, no significant difference in carcase weight was observed between treatments in either replicate. Interestingly, Warner et al. (1998) found a 12kg reduction in carcase weight of cattle on a high nutritional plane that were marketed through a saleyard compared to those directly consigned ($p = 0.06$), however, no significant liveweight change was attributed to consignment method. This suggests that the stress associated with saleyard exposure in the current study was not enough to cause physiological changes to muscle cells resulting in significant carcase weight reduction as seen in previous studies (Schaefer et al., 1997, Warner et al., 1998, Smith et al., 1982). Further, this weight loss could also be caused by inadequate water consumption/facilities in previous studies. However, although the carcase weight loss was not significant, the numerical carcase weight loss compared to the direct 24hr treatment, though minimal, should be taken into consideration.

The 14 day pathway did not gain as much weight as expected during the re-feeding period, gaining only 4.08kg and 1.37kg for Rep 1 and combined trial, respectively, from their recorded farm exit weight. Feed consumption was typical, supported by restored weight loss and the lowest level of dark cutting, implying adequate muscle glycogen at slaughter. Warriss et al. (1995) found steers transported for 5, 10 and 15 hours recovered liveweight losses of up to 7% within a 5 day period. Similarly, Earley et al. (2010) found Charolais bulls transported for up to 24 hours recovered liveweight losses of up to 7.5% within a 24 hour period with access to water and a high energy ration. Stress associated with the transition from grazing pasture to a grain based ration likely impacted liveweight gain as a result of the significant changes in the type and number of rumen microflora that occur within the first week of a ration transition (Brown et al., 2006). Feedlot cattle are generally allocated a transition period for 2 to 3 weeks from grass based to grain based rations to allow for rumen acclimatisation (Bevans et al., 2005) and thus is plausible that rumen disruption as a result of feed type transition impacted cattle performance out to 14 days. In the current study the reduced rate of weight gain post sale in 7 DAY and 14 DAY treatments is likely attributed to chronic stress, with a previous literature demonstrating the adverse impact new environment stress has on growth performance (Peterson et al., 1989, Bova et al., 2014, Endris and Feki, 2021).

5.5 Carcase characteristics

Hump height is utilised by MSA to predict primarily *Bos indicus* content of cattle which has been demonstrated to reduce eating quality through reduced tenderness associated with increased calpastatin activity (Crouse et al., 1989, Scheffler, 2022). A 10mm increase in hump height equates to an approximate 0.7 point reduction in MSA index (McGilchrist et al., 2019) with the MSA model able to predict *Bos indicus* content from hump height with 75.2% accuracy (Watson et al., 2008). Therefore, the significantly greater hump height observed in Rep 1 between the directly consigned treatment and the saleyards treatments of between 8.33 and 12.92mm may have had a small negative impact on eating quality. Consignment method has previously been found to have no effect on fat depth, however, a low plane of nutrition prior to slaughter adversely impacts fat depth in both directly consigned and saleyard cattle (P = 0.08) (Warner et al., 1998). Similarly, the present study found pathway had no significant impact on either rib fat or P8 fat depth for both replicates, although, a weak trend for a reduction in both attributes was observed in the saleyard treatments. Previous literature suggests that fat depth is only impacted after extended stress exposure or extended time off feed with nutritional stress having a minimal impact on rib fat depth in cattle fasted out to 5 days (Truscott and Gilbert, 1978). The minimal impact consignment method had on fat depth was expected given the short term exposure to stressors in the saleyard environment and the high plane of nutrition of the re-fed treatments. Animal maturity determined through ossification is known to impact palatability through its associated changes to muscle structure and subsequent adverse interaction with tenderness (Park et al., 2008, Weston et al., 2002, Shorthose and Harris, 1990). The small observed ossification range in the current study (154-170) in Rep 1 is unlikely to impact consumer palatability with an insignificant difference between treatment groups, given Bonny et al. (2014) determined a reduction of 1.8 to 2.1 MQ4 points per 100 point increase in ossification. Further, there is no evidence suggesting short term stress exposure significantly effects ossification score in cattle. On contrary, in Rep 2, the ossification range was 110–250, and the tenderness decreased by 10 points while the score increased by 150 points. Eye muscle area also had a small variation of 2.21cm^2 with no significant differences between treatment groups. This result aligns with the current understanding that short term stress exposure does not impact eye muscle area.

5.6 Dark cutting

The incidence of dark cutting in the present study is significantly higher than that recorded in the majority of previous studies (Warner et al., 1998, McGilchrist et al., 2014, Ferguson et al., 2007b). MSA determines dark cutting as carcases with a pH_u greater than 5.7 and are thus ineligible for grading under the MSA system due to negative eating quality ramifications (Thompson, 2002, McGilchrist et al., 2014). Many company requirements deem a dark cutter as a carcase with a meat colour greater than AUS-MEAT 3, however, meat colour has been shown to lack relationship to consumer eating quality score (Polkinghorne et al. 2015). Previous research shows a high correlation between meat colour and pH_u for carcases graded as dark cutting (Tarrant, 1989, Renerre, 1990), however, this was not observed in this study with at least 20 to 30% more carcases having a AUS-MEAT colour > 3 compared to a pH_u > 5.7 across all treatments. The extremely high percentage of carcases graded with an AUS-MEAT colour > 3 is likely a result of a short period of time between slaughter and grading. Hughes et al. (2014) demonstrated a significant reduction in carcases with unacceptably dark meat colour from 8 to 3% when time of grading was extended from 14 to 31 hours post slaughter (P < 0.01). The lightening of meat colour with time post-mortem is supported by the previous findings of Young et al. (1999), however, the primary mechanism of this is still unclear with possible pH interactions impacting myoglobin status and muscle structure. A possible mechanism is increased mitochondrial activity, which uses the oxygen penetrating the meat surface at the quartering site not allowing it to bind to myoglobin when there is a short time post-mortem. A recent study conducted by Cuthbertson et al. (2020) investigating the impact of stressors on dark cutting found 24% of all carcases were classified as dark cutters for pH, however, 70% were classified as dark cutters for meat colour.

The dark cutting percentages of the present study are largely affected by the small treatment size, with the number of carcases with $pH_u > 5.7$ only ranging from 3 to 5 between all treatment groups for both replicates. The proportion of carcases with $pH_u > 5.7$ was fairly consistent across the saleyard treatments indicating treatment had limited impact on muscle glycogen levels. The equal lowest levels of dark cutting and meat colour observed in the 14 day re-fed treatment for Rep 1 probably resulted from muscle glycogen being adequately replenished due to the feeding of a balanced, high energy ration prior to slaughter (Knee et al., 2007). Interestingly the direct consignment pathway had the highest proportion of dark cutting carcases (20.8%) in Rep 1 which does not align with the 12% decrease in dark cutting observed by Shorthose (1988) when cattle were directly consigned rather than marketed through the saleyard pathway, however, aligned with Rep 2 (10.8%). This dark cutting in Rep 1 may be a result of stress induced by the on-farm drafting event combined with additional room to move around in the truck pen while in transit as a result of being penned as a group of 6 on farm. Lower stocking density during transport has been found to increase movement within the pen as well as unbalance and falling (Tarrant et al., 1992) which may have resulted in glycogen depletion in the current study. Further replication of the trial is required to determine the impact of marketing pathways on dark cutting in beef cattle.

6 Conclusion

This experiment identified that marketing method significantly impacted the intrinsic eating quality of beef for the consumer. The effects of re-feeding treatment were different between the replicates. Re-feeding cattle for a period of 6 days (7 DAY) or 13 days (14 DAY) post saleyard exposure did not improve eating quality of cattle marketed through the current MSA saleyard pathway for Rep 1. There was no significant difference in MQ4 and tenderness between 48 HR and direct consignment group. However, extending the saleyard pathway out to 72 HR with access to high energy feed (1 day on feed) did not negatively impact eating quality in this cohort, with the eating quality of this treatment not differing from the directly consigned pathway. Moreover, in combined analysis, the 72 HR treatment group had all sensory qualities improved, while other treatments had numerically lower sensory scores, although not statistically significant. On the contrary, in Rep 2, the 14 DAY re-fed treatment had significantly improved consumer scores in flavour only for both the STR045 and EYE075 compared to the control 24hr treatment groups. While not statistically significant, all treatment groups in Rep 2 had improved eating quality when compared to the directly consigned treatment. Therefore, there is potential to extend the MSA saleyard pathway beyond 36 hours with the provision of water only without impacting eating quality for the consumer which also reduces the management and feed cost. Since the MQ4 and tenderness in the 48 HR group in Rep 1 were comparable to those of the direct consignment group and neither Rep 2 nor the combined trials significantly differed from the controls, the MSA saleyard pathway could be extended to a maximum of 48 hours following the current MSA saleyard pathway rules. The 48 HR pathway must involve no mixing of cattle.

Although no significant differences in consumer eating quality factors were observed between the directly consigned group and the 72-hours treatment for each replicate and the combined analysis, it was determined there was insufficient evidence (2 replicates only) to support the adoption of the 72 hours with refeed pathway. Additionally, commercial implementation of a pathway including refeeding would require additional auditing and strict processes in place at saleyards to ensure the feed quality requirements were met, along with other factors such as frequency of feeding, pen size, suitable feeding equipment and feeder access. Mob size and its impact on mixing stress would also require further evaluation.

In order to identify additional stressors that may have a negative impact on eating quality during the onsite re-feeding of cattle at saleyards, as well as the reasons behind the inconsistencies in two replicate outcomes, more replication and research should be conducted. Additionally, studies on the economic analysis of feed costs with respect to eating quality should be done.

6.1 Key findings

Combined analysis

In the combined analysis, the 72 HR re-fed treatment had improved consumer scores compared to the control treatment across all sensory attributes of MQ4, tenderness, juiciness, flavour and overall liking for both the STR045 and EYE075 cuts, although not significantly different to the other treatment groups.

The 48 HR, 7 DAY and 14 DAY re-fed treatments had a negative but non-significant relationship across all sensory attributes of MQ4, tenderness, juiciness, flavour and overall liking for both the STR045 and EYE075 compared to the direct consignment control treatment group.

Rep 1

- The average liveweights (kg) of the cattle in the 48 HR (484.9) and 72 HR (502.6) treatments were lower than their on-farm weights but the 7 DAY (517.9) and 14 DAY (537.0) re-fed cattle did not differ from their average on-farm weights (537.0).
- The average hot standard carcase weight (kg) of the cattle in the 48 HR (266.3), 72 HR (272.9) and 7 DAY (273.1) were lower compared to the 14 DAY (280.0) and the direct kill group (280.4).
- Dressing % relative to the farm exit weight appears to be greatest for the direct 24hr group (52.74%) from other treatments. The highest average dressing % in relation to the saleyard exit liveweight (54.87%) is the unfed post sale group (48 HR) which also has the lowest average dressing % relative to the farm exit weight (51.81%) reflecting reduced gut fill at saleyard exit and reduced carcase weight relative to farm weight.
- The extension of current MSA pathway up to 48 HR (36 hours + 12 hours - water only) had significantly reduced the juiciness, flavour and overall likeness for both the STR045 and EYE075 ($P < 0.05$) compared to the direct consignment group while the MQ4 and tenderness did not significantly differ.
- The 72 HR treatment (1 day on feed) was not significantly different for any consumer sensory trait or MQ4 score to the direct consignment cattle.
- The 7 DAY and 14 DAY re-fed treatments had significantly reduced consumer scores across all sensory attributes of MQ4, tenderness, juiciness, flavour and overall liking for both the STR045 and EYE075 ($P < 0.05$) compared to the direct (control 24hr) and 72 HR treatment groups, except for juiciness where the 72 HR and 14 DAY treatments did not differ.

- The impact of re-feeding on beef eating quality showed a reduction of greater than 5 points across all consumer sensory traits in the 7 DAY and 14 DAY re-fed treatments compared to cattle that were directly consigned. Surprisingly, this adverse impact was not observed in the 72 HR treatment for any consumer sensory trait or MQ4 score, implying prolonged exposure to stress has greater impact on eating quality than an initial stress event for all the meat quality attributes in replicate 1.
- Cut had a significant impact on all consumer sensory eating quality attributes ($P < 0.001$) with the STR045 eating better than the EYE075. The interaction between cut and treatment was insignificant across all sensory attributes.
- Hump height was the only carcase characteristic found to significantly impact consumer sensory eating quality attributes.

Rep 2

The direct-to-slaughter treatment (control 24hr) produced the highest hot standard carcase weight relative to farm weight result in addition to superior MSA compliance. Additionally, although not statistically significant, the dispatch that took place immediately after sale (48 hours) without feeding led to a decrease in carcase weight and an improvement in final weight as compared to the direct 24-hour group. Any period of feeding improved liveweight and hot standard carcase weight but there was no consistent relationship to days on feed.

Dressing % relative to the farm exit weight appears to be similar for all treatment groups. The highest average dressing % in relation to the sale yard exit liveweight was found for 72 HR, 7 DAY and 14 DAY treatment groups compared to the direct 24hr and 48 HR treatments.

Only the 14 DAY re-fed treatment had significantly improved consumer scores in flavour for both the STR045 and EYE075 ($P < 0.05$) compared to the other treatment groups. No significant differences were found between the control and other treatment groups across all sensory attributes scores.

6.2 Benefits to industry

The research project provides essential data to quantify the eating quality impact of alternative cattle marketing practices with four saleyard protocols compared to a direct consignment from farm to slaughter control sourced from both NSW and Victoria. It has been noted that the MSA saleyard could be increased up to 48 hours without mixing and providing water. Although the 72 HR pathway had a positive relationship with all eating quality attributes for both replications, the cattle were provided high-energy feed supplementation for 1 day. For both Rep 1 and Rep 2, the 7-DAY and 14-DAY re-fed treatments had different impacts on the sensory qualities of the beef, and results differed between replicates. Thus, it is not advisable to follow through with these re-fed methods. Additionally, it is advised that research is continued to determine why the treatment effects on the meat-eating quality attributes differed between the two replicates and is required to substantiate if there is any consistent feed, management, or environmental and genetic effect.

7 Future research and recommendations

Since the MQ4 and tenderness in the 48 HR group in Rep 1 were comparable to those of the direct consignment group and neither Rep 2 nor the combined results significantly differed from the controls, the MSA saleyard pathway could be extended to a maximum of 48 hours following the current MSA saleyard pathway rules. The 48 HR pathway must involve no mixing of cattle.

Although no significant differences in consumer eating quality factors were observed between the directly consigned group and the 72-hours treatment for each replicate and the combined analysis, it was determined there was insufficient evidence to support the adoption of the 72 hours with refeed pathway. Additionally, commercial implementation of a pathway including refeeding would require additional auditing and strict processes in place at saleyards to ensure the feed quality requirements were met, along with other factors such as frequency of feeding, pen size, suitable feeding equipment and feeder access.

Given the inconsistent meat quality results between replicates, which could lower their eating quality score from 6 to 9 points, all saleyard cattle, regardless of pathway, should be subject to the 5 point MQ4 score prediction penalty on cut x cook combinations.

Attention is required to ensure sufficient time between slaughter and grading along with stimulation to be optimised to enable correct pH/temperature declines and a genuine ultimate pH reading at grading.

Also of interest, is the consistent difference in ultimate pH between the *M. longissimus lumborum* (grading muscle) and the *M. semitendinosis*. Consistent pH differences between muscles within a carcase are considered highly likely to impact ultimate eating quality, and in particular flavour development, through interactions with muscle structure and composition, packaging, ageing, and cooking. A better understanding of these relationships could have important ramifications for MSA grading.

Additionally, it is clear that more research is needed to comprehend the management techniques, stress levels in different treatment groups and causes of the different treatment outcomes for the replicates, allowing for the widespread adoption of a particular method for enhancing meat eating quality.

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9. Appendix

Appendix I. Example (Farm A) animal tagging and drafting instruction and record sheet

RLX/MSA Saleyard Trial						**On Farm Draft**			
Property A		**Sex**							
Date					Start			Direct loaded time	
					Finish			Saleyard loaded time	
	TRIAL EARTAG		**TRIAL EARTAG**						
Order	**Number**	**Colour**	**Number**	**Colour**	**Weight**	**Direct**	**Saleyard**	**Farm Tag**	**NOTES**
1	XXXXX	XXXX	**7**	**Purple**			S		
2	XXXXX	XXXX	**8**	**Green**			S		
3	**1**	**Orange**	XXXXX	XXXX		D			
4	XXXXX	XXXX	**9**	**Yellow**			S		
5	XXXXX	XXXX	**10**	**Red**			S		
6	XXXXX	XXXX	**11**	**Purple**			S		
7	XXXXX	XXXX	**12**	**Green**			S		
8	**2**	**Orange**	XXXXX	XXXX		D			
9	XXXXX	XXXX	**13**	**Yellow**			S		
10	XXXXX	XXXX	**14**	**Red**			S		
11	XXXXX	XXXX	**15**	**Purple**			S		
12	XXXXX	XXXX	**16**	**Green**			S		
13	**3**	**Orange**	XXXXX	XXXX		D			
14	XXXXX	XXXX	**17**	**Yellow**			S		
15	XXXXX	XXXX	**18**	**Red**			S		
16	XXXXX	XXXX	**19**	**Purple**			S		
17	XXXXX	XXXX	**20**	**Green**			S		
18	**4**	**Orange**	XXXXX	XXXX		D			
19	XXXXX	XXXX	**21**	**Yellow**			S		
20	XXXXX	XXXX	**22**	**Red**			S		
21	XXXXX	XXXX	**23**	**Purple**			S		
22	XXXXX	XXXX	**24**	**Green**			S		
23	**5**	**Orange**	XXXXX	XXXX		D			
24	XXXXX	XXXX	**25**	**Yellow**			S		
25	XXXXX	XXXX	**26**	**Red**			S		
26	XXXXX	XXXX	**27**	**Purple**			S		
27	XXXXX	XXXX	**28**	**Green**			S		
28	**6**	**Orange**	XXXXX	XXXX		D			
29	XXXXX	XXXX	**29**	**Yellow**			S		
30	XXXXX	XXXX	**30**	**Red**			S		

Appendix II. Initial screen to establish CutUpDeveloper paramenters

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
634	634	Tue 14 Feb 23	Teys Wagga		24	
				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	CHK068	CHK074	CHK078	CHK081	CHK082	TFL051	TFL052	TFL064	RIB041	BRI056	BRI057	FQSHIN	HQSHIN	INT037
Direct from farm	634.1	24		L	AT	LVES						y																y																	
Group Totals of BodySides		24																																											

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	Sth Saleyard Direct

Appendix III. CUD expansion to specify muscle position(s), ageing and cooking method

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	POS1	POS2	POS3	POS4	SUM COOKS	ROTATE COOKS	GRL	LNK	SFR	THS	SC2	CRN	No. AGES	ROTATE AGES	AGE1	AGE2	AGE3	AGE4
634	634.1	Direct from farm	48	24	24		STR045	2	A1	A2			2		1	1					1		7			
634	634.1	Direct from farm	24	24	24		EYE075	1	H				1		1						1		7			

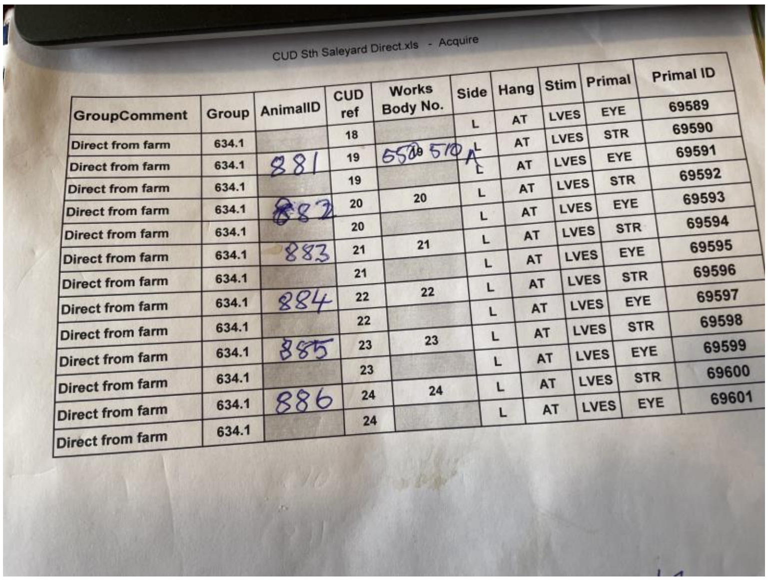
Appendix IV. Example portion of the DisplayCutUp display

SPLAYed CUT
QUIRE FINAL
e a CUT UP
E

Display of MODEL

Grp	Body	Cut	Cook	Age	Pos	Side	Cmpr	OBJ
634.1	21	STR045	GRL	7	A1	L	Ck	y
634.1	21	STR045	LNK	7	A2	L	Ck	
634.1	22	STR045	GRL	7	A1	L	Ck	y
634.1	22	STR045	LNK	7	A2	L	Ck	
634.1	23	STR045	GRL	7	A1	L	Pos	y
634.1	23	STR045	LNK	7	A2	L	Pos	
634.1	24	STR045	GRL	7	A1	L	Pos	y
634.1	24	STR045	LNK	7	A2	L	Pos	
634.1	1	EYE075	GRL	7	H	L	Ck	y
634.1	2	EYE075	GRL	7	H	L	Pos	y
634.1	3	EYE075	GRL	7	H	L	Pos	y
634.1	4	EYE075	GRL	7	H	L	Pos	y
634.1	5	EYE075	GRL	7	H	L	Pos	y
634.1	6	EYE075	GRL	7	H	L	Pos	y

Appendix V. Portion of completed Acquisition Final File



CUD Sth Saleyard Direct.xls - Acquire

GroupComment	Group	AnimalID	CUD ref	Works Body No.	Side	Hang	Stim	Primal	Primal ID
Direct from farm	634.1		18		L	AT	LVES	EYE	69589
Direct from farm	634.1	881	19	650 510 A	L	AT	LVES	STR	69590
Direct from farm	634.1		19		L	AT	LVES	EYE	69591
Direct from farm	634.1	882	20	20	L	AT	LVES	STR	69592
Direct from farm	634.1		20		L	AT	LVES	EYE	69593
Direct from farm	634.1	883	21	21	L	AT	LVES	STR	69594
Direct from farm	634.1		21		L	AT	LVES	EYE	69595
Direct from farm	634.1	884	22	22	L	AT	LVES	STR	69596
Direct from farm	634.1		22		L	AT	LVES	EYE	69597
Direct from farm	634.1	885	23	23	L	AT	LVES	STR	69598
Direct from farm	634.1		23		L	AT	LVES	EYE	69599
Direct from farm	634.1	886	24	24	L	AT	LVES	STR	69600
Direct from farm	634.1		24		L	AT	LVES	EYE	69601

Appendix VI. Section of CutUpFile relating to primals and samples fabricated from CUD Ref 1

Seq	EQS	Primal	Cut	Cook	Age	Pos	Kill	Obj	Check
AUS141022	9K5G	69554	STR045	GRL	7	A1	Tue 14 Feb 23	y	
AUS141070	0T0E	69554	STR045	LNK	7	A2	Tue 14 Feb 23		
AUS141023	9P2E	69555	EYE075	GRL	7	H	Tue 14 Feb 23	y	

Appendix VII. Section of CutUpLabels relating to samples fabricated from Primal 69554

69554	IMF 69554 - STR045 9K5G 2102	AUS141022 9K5G 69554 GRL A1 STR045 2102
AUS141070 0T0E 69554 LNK A2 STR045 2102	69555	IMF 69555 - EYE075 9P2E 2102
AUS141023 9P2E 69555 GRL H EYE075 2102		