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Evaluation of factors contributing to the incidence dark cutting in grain-fed cattle

Experiment 2: Slaughter chain audit

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Abstract

The aim of this study was to evaluate the effect of production, climate, animal, lairage and processing factors that impact the incidence of MSA pH non-compliance in 142,228 grain-fed carcasses. This value chain survey was conducted over a one year period (September 2017 to August 2018) analysing datasets from seven feedlots and three processors which recorded an average incidence of MSA pH non-compliance of 2.8%. The collation of feedlot, weather, trucking, lairage, and processing data was a huge undertaking, allowing the analysis of factors associated with MSA pH non-compliance. The production factors which caused increased pH non-compliance were producer, sex (females), cattle with HGPs, cattle morbidity, and those with longer days on feed. Processing plant, grader, increased lairage time, increased time off feed and reduced time between slaughter and MSA grading all increased the incidence of pH non-compliance. MSA pH non-compliance was also increased by lower solar radiation, lower wind speeds, higher temperatures, more rain, higher average temperature humidity index (THI) and more hours spent above heat load index (HLI_{86}) in the week before consignment. While increasing wind speeds and rain in lairage at the processor increased pH non-compliance. Heavier carcasses with whiter fat, larger hump heights, more rib fat, higher marble scores and lower ossification also have lower incidences of pH non-compliance. The results from this study suggest that minimising time in lairage and maximising time between slaughter and grading are the 2 major ways to reduce MSA pH non-compliance and high meat colour. Minimising time in lairage reduces the exposure time to stressors and reduces their ability to cause glycogen depletion, while maximising time from slaughter to grading (>20 hours) will minimise the amount of false positive pH-non-compliance and reduce incidence of high colour scores >3. A re-grading strategy could be adopted by all processors that grade carcasses <20 hours post-mortem.

Executive summary

MSA pH non-compliance beef reduces meat quality, thus beef producers are generally penalised from processors to compensate for reduced saleable quality. MSA pH non-compliance beef has been estimated to cost the Australian beef industry approximately $55 million per year. Assuming an HCWT of 280 kg the loss per carcass due to high pH non-compliance is $162.44 per carcass for the processor (Experiment 3 results). High pH meat is caused by low concentrations of muscle glycogen at slaughter which is the result of glycogen concentration on farm minus the amount used during the pre-slaughter period. The measurement time of pH post slaughter is also an important factor, Murray 1989 showed that a decreasing the time between slaughter and pH measurement at grading resulted in high pH results (pH>5.7) and dark colour. The causes of beef failing pH-compliance are multifactorial and interactions between feedlot management, transport, lairage, climate conditions and processing may have a cumulative impact on the incidence of MSA pH non-compliance. This study was undertaken to develop a comprehensive understanding of the factors that influence MSA pH non-compliance and AUS-Meat colour of grain-fed beef cattle in Australia.

The study investigated data from three processors and seven of their suppling feedlots over a 12 month period (September 1, 2017 to August 31, 2018) to identify risk factors associated with MSA pH non-compliance and AUS-Meat Colour. All three processors had a minimum of two suppling feedlots, additionally there was a feedlot that supplied two processors. Climatic data were obtained from onsite weather stations at feedlots and processors including weather stations managed by Katestone Environmental. Carcase feedback data from all producers was sourced from the Meat Standards Australia (MSA) database for statistical analyses. There were 142,228 head of cattle consigned between the three processors. For the 12 month period, intensive lairage data was collected on a monthly basis at two abattoirs however for the period between January and March 2018 lairage data were collected fortnightly.

Plant was significant in the study and the odds of carcases being classified as non-compliant based on pH at Plant B were 3.66 times the odds of Plant A ($P < 0.001$). There were no differences in likelihood of different odds of pH non-compliances occurring at Plant C in comparison to Plant A ($P = 0.561$). Grader effect was significant in the model, however was removed so the error could absorbed under processor effect. This was due to some graders having very few numbers of carcasses graded plus the same method and equipment used to assess each carcass. However the pattern of pH measurements was not very consistent across graders. There was also an extremely strong unrealistic relationship between pH and colour at Plant B and C, i.e. No pH fails on a colour score of ≤ 3 and no pH passes on a colour score ≥ 4. At plant C there were also no carcasses with a pH > 6.

Producer effect on pH non compliance was significant within the base model. Cattle supplied by Producer D were 2.21 times the odds for being non-compliant based on high pH when compared to cattle supplied from Producer A ($P < 0.001$). Inversely cattle supplied from Producer F were 0.512 times the odds less likely to be non-compliant due to high pH when compared with Producer A ($P = 0.001$). The odds of carcasses being classified as non-compliant based on pH from Producer C, Producer B or Producer G were not different from Producer A. Cattle implanted with HGP were 2.29 times the odds for being non-compliant based on high pH when compared to cattle that were HGP free ($P < 0.001$). Days on feed had a significant effect on pH compliance in the model ($P < 0.001$).

Increasing DOF was associated with a slightly higher incidence of the carcass being classified as non-compliant based on high pH. An additional 10 days on feed increased odds of high carcass pH by 1.0199 times. Males also had lower incidences of MSA pH non-compliance than females. Cattle treated for illness were significant when included in the model ($P < 0.001$) and were associated with an increase in failing on pH. If an animal was treated for illness at the feedlot, its odds of failing on pH was 1.340 times the odds of an animal that was not treated. This was expected as treated animals are exposed to increases in handling and external stimuli and therefore become more excitable, stressed and utilise more glycogen (Warriss 1990).

Weather was significant in the data however had an overall small effect on the odds ratios for cattle failing on pH. Hotter conditions over the week before exiting the feedlot was associated with small increases in pH non-compliance. This was reflected by increasing average temperature, increasing temperature humidity index and increasing the amount of hours above HLI 86. Increased average rainfall in the week before feedlot exit increased the likelihood of failing on pH. Increasing average wind speed across the 7 days before feedlot exit also decreased the odds of pH non compliance. Interestingly the weather conditions at processors for day of arrival has some significant impacts on pH non compliance. Wind speed and rainfall influenced the odds of carcasses of pH non-compliance. There was no relationship between relative humidity, ambient temperature, solar radiation or THI and odds of pH non-compliance within this model. As average wind speed increases on day 0 by 1 m/s, the odds of cattle failing on pH were 1.0288 ($P < 0.05$) times the odds of cattle failing when exposed to 1 m/s less. Rain on the day of arrival to the processor had a significant effect on the incidence of pH non compliant carcases. Rain on day 0 increased the likely hood of cattle failing on pH by 1.23 times the odds of cattle failing exposed to 1 mm total rain less.

Time in lairage had a significant effect on the incidence of MSA pH non-compliance and high colour. Animals that spent more time in lairage had an increased risk of pH non-compliance and high colour. Time to carcass grading influenced the likelihood of both pH non-compliance and high colour. Time to grade was categorised into six categories, encompassing 4 hour periods between 8 and 24 h, 24 to 48 h and then ≥ 48 h. As time to carcass grading increased past time category 1 (8 to 12 h) the odds of a carcass being classified as pH non-compliant decreased. Time between slaughter and grading had differing effect sizes on both high pH and high colour at grading. The reduced odds of having high colour with increased time to grading was greater than the reduction in odds of having high pH, with 12-16h (0.5401, 0.6910), 16-20h (0.6573, 0.9497), 20-24h (0.5310, 0.6984), 24-48h (0.2991, 0.4147) and 48+h (0.2025, 0.6450) respectively (P<0.001). However there was no difference in the odds of being non-compliant between time category 8-12 h and 16-20 h ($P = 0.514$). Plants are likely grading too early for some carcasses and the incidence of pH non-compliance and high colour would reduce if grading later (at least 20h +) or a re-grading strategy was used. Additionally the increased benefits seen for increased time to grading for a reduction in high colour show an interaction with mechanisms not directly related to pH decline. The decline in pH of carcasses has been shown to still occur up to 30h post mortem.

There were numerous carcass factors that had an effect on the incidence of pH non-compliance for the cattle in the dataset. Hot standard carcass weight influenced the incidence of pH non-compliance, where a 10 kg increase in HSCW decreased the odds of high pH 0.919 times the odds of a carcass 10 kg lighter ($P < 0.001$). Ossification was significant in the model ($P < 0.001$). As ossification score increased by 10, the odds were 1.0548 times the odds of a carcass failing on pH with an oss score 10 units lower, hence as physiological age increased there were greater odds of failing on pH.

Marbling was expressed categorically by group rather than a continuous absolute score in the base model and had a significant effect on the pH compliance of carcasses ($P \leq 0.001$). Relative to the low marbling group (100-300), the other marbling groups exhibit lower odds of failing as marbling score increased. Rib fat was significant in the model ($P < 0.001$) and had a positive effect on reducing pH non-compliance. As rib fat increased by 1 mm the odds of an animal failing on pH was 0.844 times the odds of an animal failing with 1 mm less. Hump height was also significant in the model ($P < 0.001$), as hump height increased by 10 mm the odds of a carcass failing were 0.950 times the odds of failing for a hump score of 10 mm lower. Fat colour was significant in the model ($P < 0.001$) with the odds of fat colour groups 1, 2, 3 and 4+ being 1.972, 2.465, 4.076 and 5.362 times more likely to fail on pH than the odds of fat colour group 0. Relative to fat colour 0, animals with higher fat colours tend to be increasingly more likely to have high pH. This may be attributed to being in a state of ketosis or were in a state of ketosis prior to entering the feedlot.

Greater periods in lairage allow for greater levels of stress and increase pH non-compliance. Management across the supply chain should aim to have cattle in lairage for as short a time as possible (minimum of 2 hours) as this will reduce the opportunity to be stressed and subsequently reduce the incidence of pH non-compliance. Same day kills would be ideal however the ante mortem inspectors need to be available throughout the day to assess arriving cattle. Longer times between kill and grading corresponded to a decrease in the incidence of pH non-compliance, therefore grading at a minimum of 20 hours post mortem is ideal to give carcasses every opportunity to get pH below 5.7. This will reduce the amount of high pH false positives and minimise losses to producers and processors. If processors are unable to grade after this window due to constraints, a re-grading strategy should be developed for carcasses that fail.

While the impact of weather was relatively small, high temperatures increase the likelihood of pH non-compliance. Considering the changing global environment, heat load mitigation opportunities will benefit producers in future years. However the industry must also adopt a system to alert lot feeders to identify cattle that are at greater risk of pH non-compliance so that management strategies can be implemented to minimise time in lairage and maximise time to grading.

Data management, collation and integrity needs improvement across the supply chain. It needs to be easier for data captured on cattle at the producer and processor levels to be merged and compiled to allow better performance tracking and feedback by individuals in the value chain. A centralised database capable of receiving data from multiple levels of the supply chain, verify the integrity of the data and integrate it in a way that stakeholders can produce meaningful reports and assess outcomes would be beneficial.

Plant graders were significant in the base model before removal, inconsistencies between graders should not occur. Carcass data collection needs to be as objective and accurate as possible as it underpins the entire MSA system. Not correctly using equipment such as pH meters and temperature probes, grading pH based on colour alone or rounding off on measurements decreases the correlation between recorded and biological actual.

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1 Background

MSA pH non-compliance in beef reduces meat quality, thus beef producers are generally penalised from processors to compensate for reduced saleable quality. Non-compliant pH MSA beef has been estimated to cost the Australian beef industry approximately $55 million per year (Jose *et al.* 2015). McGilchrist *et al.* (2012) estimated for 2009 the cost of non-compliant MSA beef to producers equated to approximately $0.50 (AUD) per kg carcass weight or $7.09 (AUD) for every carcass graded within the Meat Standards Australia (**MSA**) system.

In Australia, dark cutting is defined via two methods carcasses with i) a AUSmeat meat colour > 3 and/or ii) an ultimate pH > 5.70. Dark cutting, is a complex multifactorial problem that is influenced by numerous pre -slaughter factors which generate stress and exercise. The condition is generally attributed to low muscle glycogen stores at slaughter (Tarrant 1989), which is predominantly a function of glycogenesis (Loudon *et al.* 2018), minus the quantity of glycogenolysis during the pre-slaughter period. Muscle glycogenolysis has been associated with numerous factors including, but not limited to, nutritional status (Knee *et al.* 2004), particularly in grazing systems (McGilchrist *et al.* 2012; McGilchrist *et al.* 2014); water supply and quality (Loudon *et al.* 2018); animal temperament (Voisinet *et al.* 1997a; Voisinet *et al.* 1997b); sex (Voisinet *et al.* 1997a; Voisinet *et al.* 1997b); climatic conditions and climatic variability (McGilchrist *et al.* 2014)

Grain fed cattle are typically on a higher plane of nutrition compared to grass fed cattle, thus have greater glycogen stores and therefore generally lower incidence of dark cutting (Warner *et al.* 1988; McGilchrist *et al.* 2012)**.** Further speculation is that grain fed cattle are more acclimated to various stressors; including trucking, machinery, and regular contact with humans. The 2015 Australian Beef Quality audit conducted by MSA determined that the incidence of dark cutting in grain-fed cattle ranged between 1.5 % and 2.5 %, with a peak incidence occurring in March (2.5 %). Financial penalties applied to grain-fed beef range between 25 and 120 c/kg for carcasses classified as dark cutters (Jose *et al.* 2015). Whilst the incidence of dark cutting in grain-fed beef is low, Australia's total feedlot capacity is approximately 1.3 million head, however total number of grain-fed carcasses for the year ending December 2018 was 2 988 292 (Camm and McIntosh 2019). Based on these carcass numbers with an average deduction of 59 c/kg (Jose *et al.* 2015) the cost of dark cutting to feedlots ranges between 8.7 and 14.5 million, based on an average carcass weight of 330 kg, depending on the incidence of dark cutting during an annual cycle. Although it is important to consider that this cost will vary depending on current market grid prices and carcass weight. Thus dark cutting is an important factor influencing the economic viability of the feedlot industry.

The 2015 Australian beef quality audit suggests that there may be an increased incidence of dark cutting in feedlot cattle during summer, however this is not reflected in the 2017/2018 audit. This increased incidence may be associated with various factors including:

i) Climatic conditions, particularly heat load
ii) Feedlot infrastructure and management, i.e. hormone growth promotants, cattle pulls from home pen
iii) Transport conditions, i.e. dispatch time and duration of transport
iv) Lairage conditions, i.e. arrival time, time in lairage, shade, pen conditions
v) Post slaughter management, i.e. time to grading and carcass traits

Furthermore there may be interactions between feedlot management, transport, lairage and climatic conditions which may have a cumulative impact on the incidence of dark cutting. This study was undertaken to develop a comprehensive understanding of the factors that influence dark cutting of grain-fed beef in Australia. The study investigated data from three processors and seven of their suppling feedlots over a 12 month period (September 1, 2017 to August 31, 2018) to identify risk factors associated with dark cutting.

2 Project objectives

2.1 Objectives

1. Conduct a 12 month slaughter chain audit of grain-fed cattle at a minimum of three processors with a known incidence of dark cutting, and their supplying feedlots, to determine factors contributing to variation in dark cutting.
2. Describe recommendations to minimise the incidence of dark cutting carcasses in Australia.

2.2 Outcomes

1. A greater understanding of the national incidence of dark cutting in grain finished cattle and the seasonality of dark cutting
2. Expanded knowledge about the impact of climate, animal and lairage factors on the incidence of dark cutting
3. Increased profitability for producers through increased compliance to meat colour and pH requirements of slaughter grids

3 Methodology

3.1 Data sourced

Data were obtained from three processors and seven of their suppling feedlots. All three processors had a minimum of two suppling feedlots, additionally there was feedlot that supplied to two processors. Three processors and seven feedlots were approached in consultation with MLA and ALFA, to arrange their involvement in this study. Processors were defined as Plant A to C and feedlots were defined as Producer A to G.

The supply chains were as follows:

Plant A: Producers D and E

Plant B: Producers A, C and G

Plant C: Producers B, C and F

3.1.1 Weather Data

This study was conducted over a one year period analysing datasets from seven feedlots and three processors. Climatic data were obtained via three sources:

1. Onsite weather stations (Davis Pro V2, Davis Weather Station, Hayward, CA, USA)
2. Weather stations with data managed by Katestone Environmental
3. Weather data obtained from the nearest Bureau of Meteorology weather station.

An additional weather station was installed at each processing facility (n = 3) to capture onsite climatic conditions. These stations were located as close to lairage as possible without being obstructed by shade and surrounding structures that may interfere with climatic variables.

From the meteorological data, temperature humidity index (**THI**), heat load index (**HLI**) and accumulated heat load (**AHL**) for a reference animal were calculated. These were calculated from the raw weather data for the onsite stations at all processors, and some feedlots as well as the Katestone data from the remainder of the feedlots. While the Katestone weather data already included these calculated outputs, it was re-calculated again from the raw Katestone data to ensure uniformity across all weather data. The reference animal as described by Gaughan *et al.* (2008), as a clinically healthy black Angus steer < 100 days on feed.

The THI can be calculated using the following equation as adapted from Thom (1959);

$$THI = 0.8 \times T_A \left[\left(\frac{RH}{100} \times (T_A - 14.4) \right] + 46.4$$

Where RH = Relative Humidity (%) and T_A = wet bulb or dew point temperature

The HLI was calculated using the equation described by Gaughan *et al.* (2008) where the HLI equation takes the following forms;

i) A nonlinear regression which applies when BGT is greater than 25 °C

$$HLI_{BGT>25} = 8.62 + (0.38 \times RH) + (1.55 \times BGT) - (0.5 \times WS) + [e^{2.4-WS}]$$

ii) A linear model which applies when BGT falls below 25 °C;

$$HLI_{BGT<25} = 10.66 + (0.28 \times RH) + (1.3 \times BGT) - WS$$

Where RH = Relative Humidity (%); BGT = Black Globe Temperature (°C); WS = wind speed (m/s); and e = the base of the natural logarithm (approximate value of e = 2.71828)

To calculate accumulated heat load, Gaughan et al. (2008) established the following equations

i) If [$HLI_{ACC} < HLI_{Lower\ Threshold}$, ($HLI_{ACC} - HLI_{Lower\ Threshold}$)/M]; and

ii) If [$HLI_{ACC} > HLI_{Upper\ Threshold}$, ($HLI_{ACC} - HLI_{Upper\ Threshold}$)/M, 0]

Where HLI_{ACC} = the actual HLI value at a point in time; $HLI_{Lower\ Threshold}$ = the HLI lower threshold where cattle will dissipate heat (e.g. 77); $HLI_{Upper\ Threshold}$ = the HLI upper threshold where cattle will gain heat (e.g. 86); and M = number of measures per hour, i.e. number of times HLI data are collected per hour; If every 10 minutes, then M = 6 (Gaughan *et al.* 2008).

3.1.2 Meat Standards Australia carcase data

Carcase feedback data from all producers was sourced from the Meat Standards Australia (**MSA**) database for statistical analyses. Carcass data were obtained for the period 01/09/2017 to 31/08/2018 (inclusive). For this data there were a total of 142,228 head of cattle consigned across the three processors.

The MSA database data was provided in a single CSV files, with a total file size of 220MB, containing 62 columns of data for the 142,228 carcasses. When aggregating the data, the following variables were identified and established within the dataset for each carcass:

- Number of animals (n_animal) = number of animals in each lot
- Grading date (gradedate) = the date of carcass grading
- Carcase identification (bodyno) = the processors body number for each carcass
- Grader identification (grader) = the graders identifier
- Carcase hanging method (hang) = the hang method
- Hormone growth promotant status (hgp) = HGP status (Yes or No)
- Carcase sex (sex) = Steers (Male) or Female)

Carcase measurements are taken by graders accredited with both MSA grading and AUS-MEAT chiller assessment (Meat Standards Australia 2007). The carcase measurements include:

- Hump height, which is measured in 5 mm gradients and is primarily used to verify the tropical breed content declared on the vendor declaration (Meat Standards Australia 2007).
- Fat colour, is determined from the intermuscular fat lateral to the rib eye muscle. It is assessed on the chilled carcass and scored against the AUS-MEAT fat colour reference standards (AUS-MEAT Limited 2005), this is not an MSA requirement but is recorded by the processor
- Meat colour, is the predominant colour of the rib eye muscle (*longissimus thoracis et lumborum*). It is measured on the chilled carcass at the bloomed rib eye muscle face and is scored against AUS-MEAT colour reference standards (AUS-MEAT Limited 2005). Meat colour has a scale of 1 to 7, with carcasses in the range of 1B to 3 acceptable for MSA.
- MSA Marbling score, is a measure of the fat deposited between individual fibres in the rib eye muscle ranging from 100 to 1100 in increments of 10. Marbling is assessed at the quartering site of the chilled carcass and is calculated by evaluating the amount, piece size and distribution of marbling in comparison to the MSA reference standards (Romans *et al.* 1985; AUS-MEAT Limited 2005; Meat Standards Australia 2007)
- Rib fat depth (mm), is the depth of subcutaneous fat measured at the quartering site in the chilled carcass approximately 75 % of the way along the rib eye muscle (AUS-MEAT Limited 2005)
- Ossification score, is measured following the guidelines from the United States Department of Agriculture (Romans *et al.* 1985). Ossification provides a scale between 100 and 590 in increments of 10 for MSA which is an assessment of physiological age of a bovine carcass. It is a measure of the calcification in the spinous processes in the sacral, lumbar and thoracic vertebrae (AUS-MEAT Limited 2005).
- Ultimate pH (**pH_u**) and loin temperature, is measured in the rib eye muscle (*longissimus thoracis et lumborum)* of the chilled carcass at the quartering site approximately 12-18hrs post-mortem. Temperature and pH are measured using an MSA approved TPS MC-80 or TPS WP-80M ph Meter (TPS Pty Ltd., Springwood, Brisbane, Qld, 4127, Australia). pH and temperature probes should be inserted into the muscle in close proximity to each other with enough time allowed for reading to be stabilised. MSA grading cannot commence if the loin temperature is above 12 °C (AUS-MEAT Limited 2005).
- Hot standard carcase weight (**HSCW**), measured at the end of the slaughter chain in kilograms with carcases dressed to AUS-MEAT carcase standards (AUS-MEAT Limited 2005)
- Eye muscle area (**EMA**), is measured using the AUS-MEAT EMA standard grid as the number of square centimetres of *longissimus thoracis et lumborum* at the quartering site (AUS-MEAT Limited 2005), this is not an MSA requirement but is recorded by the processors

3.1.3 Feedlot data

Feedlot data sets consisted of all data captured through animal data management systems used at individual feedlots (n = 7). Feedlots either used an integrated data management or 'in-house' systems. Four feedlots used the integrated data management system provided by industry company Management for Technology. Data were amalgamated from these four feedlots. However, it is worthwhile noting that information pertaining to each lot was still needed to be individually identified and downloaded from this database.

For the remaining three feedlots data were provided via 'in-house' data management systems. Subsequently, these feedlots provided data relevant to the cattle within their lots during the study were provided in a series of spreadsheets. As expected these data files contained large variations in data identification and arrangements. This required extensive data management to enable data alignment and collation across the seven feedlots. The variable and column names then were changed to match Animal Health Data's before merging into one file per lot to be merged into one dataset. The quantity of different data sources plus the inconsistency in naming of columns with the same data is confounding and suggests an opportunity to streamline data management practices that could potentially provide opportunities for greater use in data analysis.

The feedlot variables obtained within the study included:

- Lot no: Number assigned to a group of cattle
- NLIS: National livestock Identification System number for each individual animal.
- Entry weight: First recorded weight of an individual animal on entry to feedlot at induction
- Exit weight: Last recorded weight on an individual animal before or on exit date.
- Induction date: Date cattle entered feedlot
- Exit date: Date cattle exited feedlot
- Average daily gain (ADG): The average daily gain in kgs for an animal over the feedlot period. Calculated by (exit weight – entry weight) / Days on feed (there is inherent error in the ADG term as some cattle are inducted when empty whilst others have been on feed for a few days but the fed state of cattle is not recorded so it cannot be accounted for in the ADG term)
- Days on feed (DOF): The total amount of days between induction date and exit date.
- Pull true (Yes/No): If an animal was removed to the hospital pen for reasons such as injury, illness, or shy feeding.
- Exit time: Exit time from feedlot. Taken from the National Vendor Declaration forms sent to processor with each lot of cattle. These were then recorded into excel from either photographs or scanned copies of the NVDS. These were lined up to MSA feedback using the variables kill date, feedlot and number of head.
- Arrival time: Arrival time of cattle to the processor. Taken from photographs or scans of hand written trucking sheets for groups of cattle entering plant. These were lined up to MSA feedback using the variables kill date, feedlot and number of head. Often the number of head on the trucking sheets were incorrect, this could be rectified by searching individual animal NLIS numbers from feedlot data and matching to MSA feedback to find lot numbers but was extremely time consuming.
- Time in Lairage: (kill time – arrival time)

- Time off feed: Time in lairage + transport time
- Transport time: Arrival time – Exit time

Cattle DOF were determined from the producer data, as data obtained from the MSA database reporting of DOF were recorded incorrectly (Figure 1).

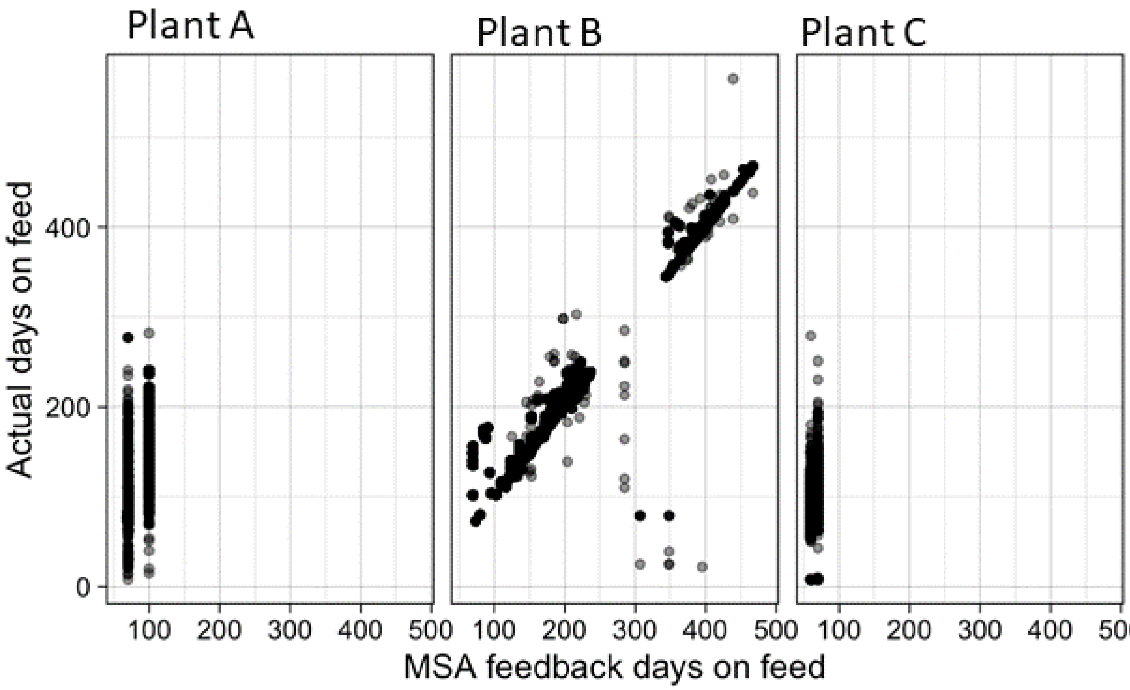

Figure 1: Variation in days on feed from the producers in comparison to data obtained from the Meat Standards Australia (MSA) carcase database

3.1.4 Intensive lairage data

For the 12 month period, between September and August, lairage data was collected on a monthly basis at two processors. However for the period between January and March 2018 lairage data were collected fortnightly at two abattoirs. Data collection was conducted for periods of three to five days for each collection period. These observations were collected at approximately 14 ± 6 day intervals. In total 30 intensive lairage observations were conducted within the study, 15 per abattoir. Data collected during these observations included:

- Provision of shade
- Washing at processor
- Panting score, percentage of lot, as described by Brown-Brandl *et al.* (2006), Mader *et al.* (2006) and Gaughan *et al.* (2008).
    - Panting scores were initially evaluated on arrival and then one hour after unloading
- Ease of unloading
- Number of slips and/or falls during unloading
- Stocking density in lairage pens (kg carcass weight/m^2)
- Number of washing events
- Number of pen relocations during lairage
- Electrical stimulation: Electrical stimulation protocols were standard within each plant across all days for the 12 months of data collection, the inputs are described in Table 3.2 of Experiment 3 – MLA Project B.FLT.0399. The average time of stimulation for each

operational unit was measured by the project personnel. Fifty carcasses were randomly selected each day on site during visits from researchers. PH declines were conducted on these bodies as per AUSMEAT standards to ensure carcasses were passing through pH window.

3.1.5 Statistical Analysis

All data management and analysis was performed in R (R Core Team 2018). Data merging and manipulation was performed using the 'dplyr' package (Wickham et al. 2019), whilst exploratory visualisations were generated within the 'ggplot2' package (Wickham et al. 2019) and summary tables were generated using the package 'table1' (Rich 2018). In addition calendar plots summarising weather data were generated using the 'sugrrants' package (Wang et al. 2019). Time series manipulations were conducted utilising the 'tsibble' package (Wang et al. 2019).

Data was merged from various sources as previously described. Where possible, data were merged at an individual animal level via their unique national livestock identification systems (NLIS) identifier. For the lariage data, lot level information merged by kill date, plant and lot size.

The baseline models, with an indicator variable pH fail as the dependent variable, were fit as generalised linear models with a logistic link function, such that the estimated coefficients may be interpreted as log-odds (or odds ratios when exponentiated). Where appropriate, plant and producer were always included as main effects. pH has been used as the dependent variable because the meat colour data was deemed to be not as reliable (see final report for B.FLT.0399 experiment 3).

The weather models were fit using generalised linear mixed models using the 'lme4' package (Bates et al. 2019). Unfortunately there were some corruptions with on-site weather stations, thus weather data herein pertains to data from the following producers: Producer B, Producer C, Producer D and Producer G. Three models were evaluated to investigate the relationship between climatic conditions during the 7 days prior to feedlot exit and non-compliant pH MSA in beef carcasses. Within each of the models sex and HGP status were included as fixed effects and producer and kill date as random effects. The modes were:

Model 1. Solar radiation (W/m^2), wind speed (m/s), rain (mm), relative humidity (%) and ambient temperature (°C)
Model 2. Solar radiation, wind speed, rain, humidity and temperature humidity index (THI)
Model 3. Rain and heat load index (HLI).

The climatic conditions were evaluated using three separate models as there are inherent interrelationships between climatic variables and climatic indices, specifically the HLI is a function of black globe temperature, relative humidity and solar radiation. Furthermore, data evaluated within Model 3 is not directly comparable to the above models as there were fewer observations that had HLI values. Within Model 3 a HLI threshold of 86 (HLI_{86}) was used to evaluated the relationship between HLI and non-compliant pH . A HLI value of 86 is a representative value of a reference animal that was defined by Gaughan *et al.* (2008) a healthy un-shaded Angus less than 100 days on feed.

Model outputs were visualised and tabularised with the 'sjPlot' package (Lüdecke 2019). This includes the forest plots for coefficients and tables of estimated odds ratios. Posthoc pairwise difference estimates were found using the 'emmeans' package (Lenth 2019). There were some variables that were confounded within the dataset and as such have been excluded from the statistical analysis, however these have been described within Appendix 9.1.

4 Results

The baseline model (Table 1), with an indicator variable pH fail as the dependent variable. Variables were fit as generalised linear models with a logistic link function, such that the estimated coefficients may be interpreted as log-odds (or odds ratios when exponentiated). Where appropriate, plant and producer were always included as main effects. Most variables showed up as statistically significant, but this is in large associated with the sample size.

Table 1: Base model for pH fail odds ratio prediction including significant variables

Predictors	Odds Ratio	Confidence Interval	Significance
Intercept	0.1972	0.1319 – 0.2947	$P < 0.001$
DOF (10 day increments)	1.0199	1.0098 – 1.0301	$P < 0.001$
HCSW (10 kg increments)	0.9198	0.9120 – 0.9278	$P < 0.001$
HGP Status	2.2927	2.0340 – 2.5843	$P < 0.001$
Fat Colour (1)	1.9728	1.7723 – 2.1959	$P < 0.001$
Fat Colour (2)	2.4652	2.2047 – 2.7564	$P < 0.001$
Fat Colour (3)	4.0762	3.3573 – 4.9491	$P < 0.001$
Fat Colour (4+)	5.3624	3.7524 – 7.6633	$P < 0.001$
Time to Grading (12-16 h)	0.6910	0.6083 – 0.7851	$P < 0.001$
Time to Grading (16-20 h)	0.9497	0.8134 – 1.1089	$P = 0.514$
Time to Grading (20-24 h)	0.6984	0.5823 – 0.8376	$P < 0.001$
Time to Grading (24-48 h)	0.4147	0.3112 – 0.5526	$P < 0.001$
Time to Grading (48h +)	0.6450	0.5495 – 0.7572	$P < 0.001$
Hump Height (10 mm increments)	0.9504	0.9310 – 0.9703	$P < 0.001$
Rib Fat	0.8441	0.8332 – 0.8551	$P < 0.001$
MSA Marble (300-500)	0.5710	0.5216 – 0.6252	$P < 0.001$
MSA Marble (500-700)	0.6855	0.5515 – 0.8521	$P = 0.001$
MSA Marble (700+)	0.4011	0.2829 – 0.5687	$P < 0.001$
Ossification (10 score increments)	1.0548	1.0464 – 1.0632	$P < 0.001$

Sex (Steer)	1.1458	1.0361 – 1.2671	*P* = 0.008
Plant B	3.6593	2.4913 – 5.3749	*P* < 0.001
Plant C	0.8803	0.5728 – 1.3530	*P* = 0.561
Producer B	1.2664	0.8767 – 1.8293	*P* = 0.208
Producer C	0.9503	0.6827 – 1.3227	*P* = 0.762
Producer D	2.2074	1.7298 – 2.8169	*P* < 0.001
Producer F	0.5125	0.3458 – 0.7595	*P* = 0.001
Producer G	0.9752	0.7448 – 1.2768	*P* = 0.855

4.1 Incidence of pH non-compliance

4.1.1 Incidence by processor

Plant A, Plant B and Plant C had a pH non-compliance incidence of 2.86 %, 3.15 % and 2.56 % over the duration of the study (Table 2). Plant was a significant variable in the base model and the odds of carcases being classified as non-compliant MSA based on ultimate pH at Plant B were 3.66 times the odds of pH non-compliances occurring at Plant A (*P* < 0.001; Table 1). There were no differences in likelihood of different odds of pH non-compliances occurring at Plant C in comparison to Plant A (*P* = 0.561).

Table 2: Number of carcasses classified as compliant (pH ≤ 5.69) and non-compliant (pH ≥ 5.70) across the three processing facilities

Plant	Total Carcasses	Compliant	Non-Compliant	Proportion Non-Compliant
A	68 431	66 474	1 957	2.86 %
B	41 693	40 379	1 314	3.15 %
C	30 698	29 913	785	2.56 %

4.1.2 Incidence by feedlot

Producer D supplied the most cattle with 62 349 head, followed by Producer G (19,147 head), Producer C (18,989 head) and Producer A (18,546 head), respectively (Table 3). Producer G (5.61 %) had the highest incidence of non-compliant MSA followed by Producer B (3.26 %) and Producer D (3.12 %; Table 3), whereas the lowest incidence of non-compliance was observed from Producer E (1.16 %; Table 3).

Table 3: Number of carcasses classified as compliant (pH ≤ 5.7) and non-compliant (pH > 5.70) across the seven producers

Producer	Total Carcasses	Compliant	Non-Compliant	Proportion Non-Compliant
A	18 546	18 314	232	1.27 %
B	7 472	7 236	236	3.26 %
C	18 989	18 510	479	2.59 %
D	62 349	60 462	1 887	3.12 %
E	6 082	6 012	70	1.16 %
F	8 237	8 102	135	1.67 %
G	19 147	18 130	1 017	5.61 %

The influence of producer on high pH was significant within the base model (Table 1). Cattle supplied by Producer D were 2.21 times the odds for being non-compliant based on high pH when compared to cattle supplied from Producer A ($P < 0.001$). Inversely cattle supplied from Producer F were 0.512 times the odds less likely to be non-compliant due to high pH when compared with Producer A (P = 0.001). The odds of carcasses being classified as pH non-compliant from Producer C (P = 0.762), Producer B (P = 0.208) or Producer G (P = 0.855) were not different from Producer A.

4.2 Animal Factors influencing pH non-compliance

4.2.1 Influence of Hormone Growth Promotants

Hormonal growth promotants (**HGP**) were implanted in 72.7 % of the cattle captured within this study (Table 4). Producer B and Producer F had a HGP usage rate of 100 %, whereas Producer A did not use HGP in their cattle (Table 4), hence for the analysis, these three producers and HGP status are confounded. The cattle implanted with HGP had pH non-compliance incidence of 3.33 %, whereas HGP free cattle had an incidence of 1.68 % (Table 5). Cattle implanted with HGP were 2.29 times the odds for being non-compliant based on high pH when compared to cattle that were HGP free ($P < 0.001$; Table 1)

Table 4: Number of cattle implanted with Hormone Growth Promotants (HGP; %) across the seven producers during the study

HGP	Producer							Overall HGP
	A	B	C	D	E	F	G	
Yes	0 (0 %)	7 472 (100 %)	15 899 (83.7 %)	55 129 (88.4 %)	5 741 (94.4 %)	8 237 (100 %)	9 933 (51.9 %)	72.7 %
No	18 546 (100 %)	0 (0 %)	3 090 (16.3 %)	7 220 (11.6 %)	341 (5.6 %)	0 (0 %)	9 214 (48.1 %)	27.3 %

Table 5: Number of carcasses classified as compliant (pH ≤ 5.69) and non-compliant (pH ≥ 5.70) as evaluated by Hormone Growth Promotant

HGP	Total Carcasses	Compliant	Non-Compliant	Proportion Non-Compliant
No	38 411	37 765	646	1.68 %
Yes	102 411	99 001	3 410	3.33 %

4.2.2 Influence of sex

Males accounted for 71.5% of cattle within the study (Table 6). Producer B fed a higher proportion of female cattle (96.6 %), whereas Producer G (92.7 %) and Producer E (99.9 %) fed a higher proportion of male cattle (Table 6). Female and male carcasses had a total pH non-compliance incidence of 3.21 % and 2.86 %, respectively (Table 7). Although sex was significant in the model, the odds ratio for males being non-compliant based on high pH was only slightly greater than the odds for females in the base model (1.1458, $P = 0.008$; Table 1). When producer was removed from the baseline model (removing confounding of producer and sex), the males had a lower odds ratio than females, aligning with the raw data (data not shown).

Table 6: Distribution of female and male cattle fed across the seven producers during the study

Sex	Producer							Overall
	A	B	C	D	E	F	G	
Female	2 488 (13.4 %)	7 221 (96.6 %)	14166 (74.6%)	10105 (16.2%)	6 (0.1%)	4756 (57.7%)	1401 (7.3%)	28.5 %
Male	16 058 (86.6 %)	251 (3.4 %)	4823 (25.4%)	52244 (83.8%)	6076 (99.9%)	3481 (42.3%)	17746 (92.7%)	71.5 %

Table 7: Number of female and male carcasses classified as compliant (pH ≤ 5.69) and non-compliant (pH ≥ 5.70)

Sex	Total Carcasses	Compliant	Non-Compliant	Proportion Non-Compliant
Female	40 143	38 894	1 249	3.21 %
Male	100 679	97 872	2 807	2.87 %

4.2.3 Days on feed

Mean days on feed (**DOF**) across producers was 128 ± 76. Producer A had the highest days on feed with an average of 285 ± 92 days (Table 8). Producer F had the lowest average days on feed with 61 ± 3 (Table 8). Days on feed had a significant interaction in the model (*P* < 0.001). Increasing DOF was associated with a slightly higher incidence of the carcass being classified as pH non-compliant. A 10 day increased in DOF increased odds of high carcass pH by 1.0199 times (Table 1).

Table 8: The mean, median, minimum and maximum days on feed for cattle from each producer

Item	Producer						
	A	B	C	D	E	F	G
Mean	285 ± 92	82 ± 17	96 ± 36	105 ± 25	98 ± 7	61 ± 3	136 ± 38
Median	223	82.0	83.0	103	100	60.0	134
Minimum	22.0	8.0	43.0	8.0	70.0	60.0	69.0
Maximum	565	279	256	282	100	70.0	440

4.2.4 Feedlot morbidity

Feedlot morbidity was significant when included in the model (*P* < 0.001) and were associated with an increased likelihood in being classified as non-compliant based on high pH (Table 9). If an animal was identified as morbid the odds of having a non compliant high pH was 1.340 times that of a healthy animal.

Table 9: Base model for odds ratio of pH noncompliance using feedlot morbidity

Predictors	Odds Ratio	Confidence Interval	Significance
Intercept	0.1783	0.1192 – 0.2668	*P* < 0.001
Morbid	1.3409	1.2186 – 1.4755	*P* < 0.001

4.3 Weather factors influencing pH non-compliance

4.3.1 Climate Model 1. Solar radiation, wind speed, rain, relative humidity and ambient temperature

Solar radiation, wind speed, rainfall and average ambient temperature influenced the odds of carcasses being classified as pH non-compliant (Table 10). There was no relationship between relative humidity (*P* = 0.404) and ambient temperature (range, *P* = 0.905; minimum, *P* = 0. 204; or maximum, *P* = 0. 154) and odds of pH non-compliance within this model Table 10).

Cattle exposed to a higher average solar radiation during the 7 days prior to feedlot exit had a slightly lower likelihood of being classified as pH non-compliant, when compared to animals that were exposed to lower solar loads (*P* < 0.05). The odds ratio for high pH for average solar radiation was 0.997 times the odds of failing on pH if exposed to one average W/m^2 over the 7 days prior to feedlot exit. Similarly, higher average wind speeds over the 7 days prior to feedlot exit were associated with lower odds of being classified pH non-compliant ($P < 0.05$). Cattle exposed to an average wind speed 1 m/s faster over the 7 days had 0.9611 times the odds of pH non-compliance, compared with an animal with an average wind speed 1 m/s slower. An increased rainfall during the 7 days prior to feedlot exit increased the odds of pH non-compliance ($P < 0.001$), where cattle that experienced 1 mm of rain more had 1.0129 times the odds of high pH when compared to cattle that were exposed to 1 mm less rainfall within the same period. Furthermore higher average ambient temperature during the 7 day prior to feedlot exit correlated with an increase in the odds of pH non-compliance ($P < 0.05$). Cattle that were exposed to an increased average ambient temperature by 1 °C had 1.0315 times the odds of high pH compared with cattle exposed to average ambient temperatures that were 1 °C lower during the 7 days prior to feedlot exit.

Table 10: The odds ratios for the effect of temperature (mean, range, max and min), solar radiation, wind speed, relative humidity and rain on the incidence of pH non-compliance

Predictors	Mean Model		Range Model		Max Model		Min Model	
	Odds Ratio	Significance	Odds Ratio	Significance	Odds Ratio	Significance	Odds Ratio	Significance
Intercept	0.0118	$P < 0.001$	0.0394	$P < 0.001$	0.0429	$P < 0.001$	0.0152	$P < 0.001$
SR_{MEAN}	0.9970	$P = 0.013$	0.9986	$P = 0.096$			0.9980	$P = 0.061$
SR_{MAX}					0.9988	$P = 0.004$		
WS_{MEAN}	0.9611	$P = 0.022$	0.9875	$P = 0.496$				
WS_{MAX}					0.9857	$P = 0.085$		
WS_{MIN}							0.6480	$P = 0.036$
RH_{MEAN}	1.0039	$P = 0.404$						
RH_{RANGE}			0.9886	$P = 0.020$				
RH_{MAX}					0.9926	$P = 0.171$		
RH_{MIN}							1.0084	$P = 0.119$
$T_{A, MEAN}$	1.0315	$P = 0.035$						
$T_{A, RANGE}$			0.9982	$P = 0.905$				
$T_{A, MAX}$					1.0207	$P = 0.154$		
$T_{A, MIN}$							1.0151	$P = 0.204$
Rain	1.0129	$P < 0.001$	1.0140	$P < 0.001$	1.0123	$P < 0.001$	1.0135	$P < 0.001$

4.3.2 Climate Model 2. Solar radiation, wind speed, rain, humidity and temperature humidity index

Solar radiation, wind speed and rainfall influenced the odds of carcasses being classified as pH non-compliant (Table 11). There was no relationship between relative humidity ($P = 0.404$) and THI (range, $P = 0.666$; minimum, $P = 0.263$, or maximum, $P = 0.113$) and pH non-compliance within this model.

An increased in average THI during the 7 days prior to feedlot exit were associated with an increase in the odds of carcases with pH non-compliance. A one unit increase in THI had 1.0253 times the odds of having a non-compliant pH when compared to cattle that were exposed to conditions with a THI one unit lower ($P < 0.05$). Similar to Climate Model 1 an elevated solar radiation during the 7 days prior to feedlot exit were associated with a lowered the odds of carcasses having a high pH. The odds ratio for carcases being classified as pH non-compliant were 0.9969 times the odds of cattle exposed to solar loads 1 W/m^2 during the 7 days before exit ($P < 0.01$). Faster average wind speeds over the 7 day period were associated with lower odds of pH non-compliance. Cattle exposed to average wind speeds 1 m/s had 0.9621 times the odds of having high pH carcase when compared to an animal that was exposed to average wind speeds 1 m/s slower ($P < 0.05$). An increase in rainfall was associated with an increase odds of pH non-compliance. Cattle exposed to 1 mm higher rainfall had 1.0130 times the odds of high pH than cattle exposed to 1 mm less rainfall during the 7 days prior to feedlot exit ($P < 0.001$).

Table 11: The odds ratios for the effect of THI (mean, max, range and min) plus solar radiation, wind speed, relative humidity and rain on the incidence of pH non-compliance.

Predictors	Mean Model		Range Model		Max Model		Min Model	
	Odds Ratio	Significance	Odds Ratio	Significance	Odds Ratio	Significance	Odds Ratio	Significance
Intercept	0.0048	$P < 0.001$	0.0429	$P < 0.001$	0.0143	$P < 0.001$	0.0113	$P < 0.001$
SR_{MEAN}	0.9969	$P = 0.009$	0.9984	$P = 0.084$			0.9981	$P = 0.076$
SR_{MAX}					0.9987	$P = 0.003$		
WS_{MEAN}	0.9621	$P = 0.024$	0.9874	$P = 0.487$				
WS_{MAX}					0.9866	$P = 0.102$		
WS_{MIN}							0.6497	$P = 0.038$
RH_{MEAN}	1.0027	$P = 0.547$						
RH_{RANGE}			0.9888	$P = 0.019$				
RH_{MAX}					0.9916	$P = 0.131$		
RH_{MIN}							1.0088	$P = 0.104$
THI_{MEAN}	1.0253	$P = 0.017$						
THI_{RANGE}			0.9956	$P = 0.666$				
THI_{MAX}					1.0252	$P = 0.113$		
THI_{MIN}							1.0083	$P = 0.263$
Rain	1.0130	$P < 0.001$	1.0141	$P < 0.001$	1.0120	$P < 0.001$	1.0134	$P < 0.001$

4.3.3 Climate Model 3. Rain and heat load index

An increase in the number of hours above HLI_{86} during the 7 days prior to feedlot exit was associated with an increased odds of a carcass having non-compliant pH (Table 12). Cattle that were exposed to 1 h longer per day of HLI_{86} over the 7 day period were 1.0118 times the odds of having a non-compliant pH when compared to cattle that were exposed to HLI_{86} for 1 h less during the 7 days prior to feedlot exit. Interestingly average (P = 0.497), maximum (P = 0.748) and minimum (P = 0.525) HLI were not associated with an increased likelihood of pH non-compliance. Additionally, within this model rain did not influence the likelihood of pH non-compliance ($P > 0.05$).

Table 12: The odds ratios for the effect of HLI mean, max , min, $HLI_{<70}$[1] and HLI_{86}[2] plus rain on the incidence of pH non-compliance

Predictors	Mean Model		Max Model		HLI_{86} Model		$HLI_{<70}$ Model	
	Odds Ratio	Significance	Odds Ratio	Significance	Odds Ratio	Significance	Odds Ratio	Significance
Intercept	0.0105	$P < 0.001$	0.0182	$P < 0.001$	0.0137	$P < 0.001$	0.0110	$P < 0.001$
HLI_{MEAN}	1.0064	$P = 0.497$						
HLI_{MAX}			0.9980	$P = 0.748$				
$HLI_{<70}$[1]							1.9360	$P = 0.525$
HLI_{86}[2]					1.0118	$P = 0.011$		
Rain	1.0020	$P = 0.608$	1.0025	$P = 0.519$	1.0019	$P = 0.623$	1.0236	$P = 0.268$

[1]number of days where HLI did not go below 70 for equal to or greater than 6 hours during the 7 days prior to feedlot exit

[2]number of days where HLI was > 86 during the 7 days prior to feedlot exit

4.4 Lairage factors influencing pH non-compliance

4.4.1 Time in lairage

Transport time was identified as not significant (*P* = 0.927). This can be partly explained by there not being a very large range of transport times within each producer. Specifically, all of the transport times for Producer B (≈ 7 to 8 h), D (≈ 2 to 4 h), E (≈ 6 h) and Producer F (≈ 4 h) were similar for each lot of cattle (Figure 2). Producer C supplied Plants B and C and had a greater range of transport times with majority at 2 H.

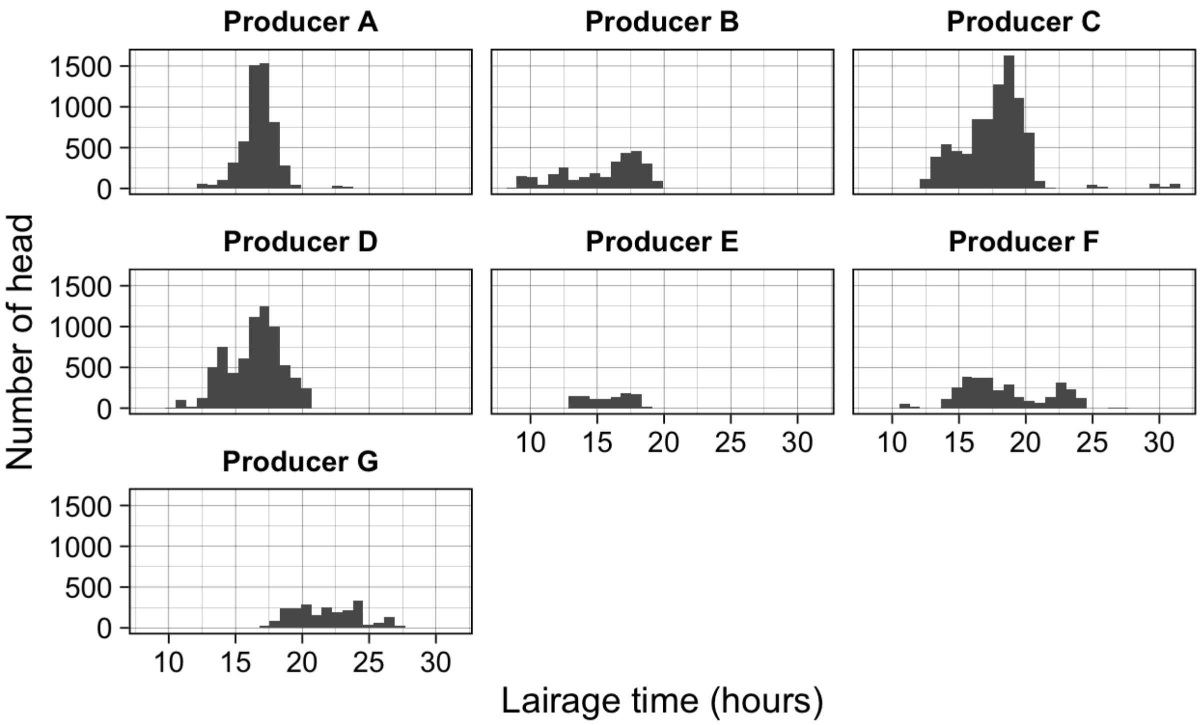

Figure 2: Approximate transport duration (hours) for all producers

Time in lairage had a greater variability (Figure 3), and was associated with increased incidence of pH non-compliance (*P* < 0.001). The odds of high pH for animals who have an additional hour in lairage are 1.06 times the odds of animals without the additional hour of lairage (Table 13). Additionally transport time and time in lairage were used to time to estimate time off feed. Time cattle were off feed was also associated with an increased incidence of non-compliant MSA (P < 0.001) and had an odds ratio of 1.06 (Table 13).

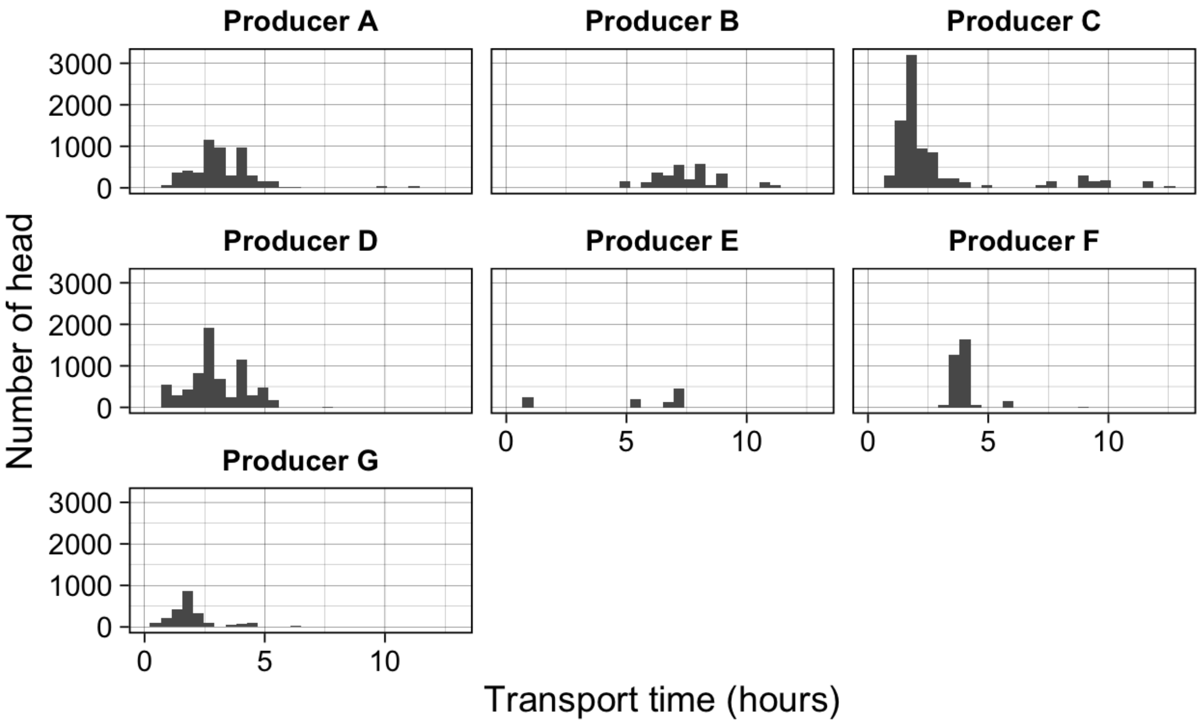

Figure 3: Approximate time in lairage (hours) for all producers

Table 13: The effect of transport, lairage and time off feed on the odds of pH non-compliance for grain fed cattle

Predictors	Baseline Model		Lairage Model		Transport Model		Time off Feed Model	
	Odds Ratio	**Significance**	**Odds Ratio**	**Significance**	**Odds Ratio**	**Significance**	**Odds Ratio**	**Significance**
Intercept	0.10	*P* = 0.087	0.03	*P* = 0.015	0.17	*P* = 0.226	0.02	*P* = 0.010
lairage time			1.06	**<0.001**				
transport time					1.00	0.927		
time off feed							1.06	**<0.001**

4.4.2 Climate conditions during lairage

During lairage wind speed and rainfall influenced the odds of carcasses being classified as pH non-compliant (Table 14). There was no relationship between relative humidity, ambient temperature or solar radiation and odds of pH non-compliance within this model. As average wind speed increases on day 0 by 1m/s, the odds of arriving cattle failing on pH were 1.0288 ($P < 0.05$) times the odds of cattle failing when exposed to 1 m/s less. A similar result was shown with wind speed min and max which had an odds ratio of 1.051 and 1.0324 respectively. Rain on the day of arrival to the processor had a significant effect on the incidence of pH non-compliance. Rain on day 0 increased the likely hood of cattle failing on pH by 1.23 times the odds of cattle failing exposed to 1mm total rain less (Table 14). Temperature humidity index was not significant in when modelled ($P > 0.05$).

Table 14: The odds ratios for the effect of temperature (mean, range, max and min), solar radiation, wind speed, relative humidity and rain during lairage on the incidence of dark cutting

Predictors	Mean Model		Range Model		Max Model		Min Model	
	Odds Ratio	Significance	Odds Ratio	Significance	Odds Ratio	Significance	Odds Ratio	Significance
Intercept	0.0116	$P < 0.001$	0.0094	$P < 0.001$	0.0108	$P < 0.001$	0.0091	$P < 0.001$
SR_{MEAN}	0.9997	$P = 0.748$	0.9996	$P = 0.576$			0.9998	$P = 0.816$
SR_{MAX}					0.9996	$P = 0.084$		
WS_{MEAN}	1.0288	$P = 0.014$	1.0268	$P = 0.034$				
WS_{MAX}					1.0324	$P < 0.001$		
WS_{MIN}							1.0513	$P = 0.009$
RH_{MEAN}	0.9977	$P = 0.571$						
RH_{RANGE}			0.9940	$P = 0.125$				
RH_{MAX}					0.9963	$P = 0.362$		
RH_{MIN}							1.0014	$P = 0.681$
$T_{A,MEAN}$	0.9897	$P = 0.336$						
$T_{A,\ RANGE}$			1.0154	$P = 0.183$				
$T_{A,\ MAX}$					1.0044	$P = 0.640$		
$T_{A,\ MIN}$							0.9971	$P = 0.742$
Rain	1.2315	$P < 0.001$	1.2213	$P < 0.001$	1.2141	$P < 0.001$	1.2266	$P < 0.001$

4.5 Processor factors influencing pH non-compliance

4.5.1 Time to grading

Time to carcase grading influenced the likelihood of MSA pH non compliance. Time to grade was categorised into six categories, encompassing 4 hour periods between 8 and 24 hours, 24 to 48 hours and then ≥ 48 hours (Table 15). As time to carcase grading increased past time category 1 (8 to 12 h) the odds of a carcass being classified as non-compliant for pH decreased. Time category 2, 4, 5 and 6 were 0.691, 0.698, 0.414 and 0.645 times the odds of time category 1 for non-compliant MSA carcasses based on high pH ($P < 0.001$) (Table 1). However there was no difference in the odds of pH non-compliance between time category 1 and 3 ($P = 0.514$) (Table 1).

Table 15: Time to carcass grading categories showing total carcasses and proportion carcasses graded within each time category

Time Category	Time to Grading, h	Total Carcasses	Proportion Graded
1	8 h to 12 h	8 179	5.81
2	12 h to 16 h	63 498	45.09
3	16 h to 20 h	19 635	13.94
4	20 h to 24 h	26 969	19.15
5	24 h to 48 h	3 456	2.45
6	≥ 48 h	19 085	13.55

4.6 Carcase factors influencing dark cutting

4.6.1 Hot Standard Carcass Weight

Hot standard carcase weight (**HSCW**) varied between producers, although mean HSCW was 332 ± 60.5 kg (Table 16). Hot standard carcase weight influenced the incidence of pH non-compliance, where a 10 kg increase in HSCW the odds of high pH was 0.919 times the odds of a carcass 10 kg lighter ($P < 0.001$). This is further emphasised when carcasses were grouped by sex and compared (Figure 4). The average HSCW was lower when carcasses had a pH > 5.7, irrespective of sex (Figure 4).

Table 16: The mean, median, minimum and maximum HSCW's for cattle from each producer

Item	Producer						
	A	B	C	D	E	F	G
Mean	424 ± 43.2	287 ± 29.5	298 ± 57.2	324 ± 35.3	333 ± 37.5	260 ± 24.4	349 ± 62.6
Median	426	288	281	324	332	259	351
Minimum	196	197	169	102	187	172	61
Maximum	694	381	554	483	471	348	602

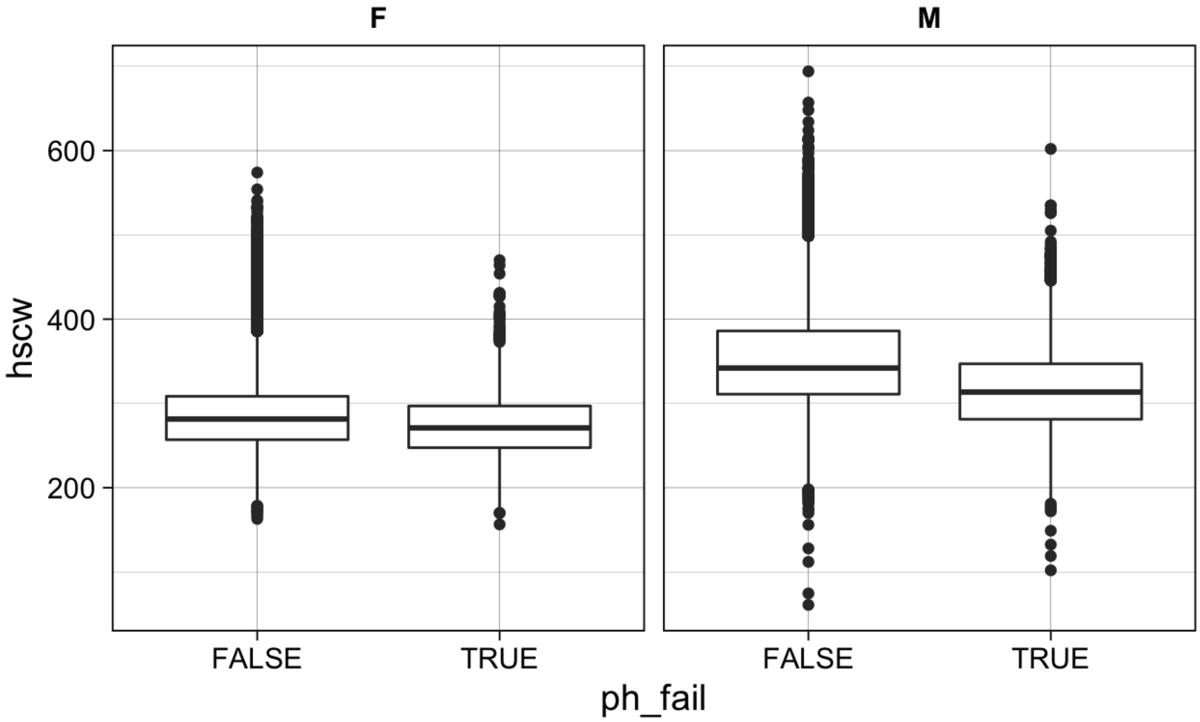

Figure 4: The mean, median, min and max HSCW's for pH> 5.71 or pH<5.70 for males and females

4.6.2 Ossification

There was some variability in ossification score across the seven producers within this study (Table 17). Ossification was significant in the model (*P* < 0.001; Table 1). As ossification score increased the odds of the carcass being classified as pH non-compliant, where and increase in ossification score by 10 units the odds of non-compliant MSA pH were 1.0548 times the odds compared with a carcass with an ossification score 10 units lower.

Table 17: The mean, median, minimum and maximum ossification scores for cattle from each producer

Item	Producer						
	A	B	C	D	E	F	G
Mean	168 ± 55.5	159 ± 28.6	154 ± 23.4	153 ± 18.4	151 ± 13.2	143 ± 16.9	163 ± 37.4
Median	160	150	150	150	150	140	160
Minimum	100	110	100	100	100	100	100
Maximum	590	400	400	500	250	280	590

4.6.3 Marbling

Producer A had the highest mean marble score with 619 ± 227, however on average marble scores across producers was 387 ± 140 (Table 18).

Marbling was evaluated categorically by group rather than a continuous absolute score in the base model and had a significant effect on the pH compliance of carcasses. Marbling was significant in the model for the 300 to 500 ($P < 0.001$), 500 to 700 ($P = 0.001$) and > 700 ($P < 0.001$) marbling categories (Table 1). The odds of each marbling group having a high pH, in ascending marble score, were 0.571 (MSA Marble 300 to 500), 0.685 (MSA Marble 500 to 700) and 0.401 (MSA Marble 700+) times the odds of when compared with the lowest MSA marbling category (MSA Marble 100 to 300). Relative to the low marbling group (100-300), the other marbling groups exhibit lower odds of being classified as non-compliant MSA pH as marbling score increased.

Table 18: The mean, median, minimum and maximum MSA marble scores for each producer

Item	Producer						
	A	B	C	D	E	F	G
Mean	619 ± 227	380 ± 56	392 ± 72	351 ± 54	353 ± 48	358 ± 52	301 ± 113
Median	560	360	360	350	350	350	300
Minimum	100	210	120	100	100	180	100
Maximum	1 190	590	980	1000	780	600	1050

4.6.4 Rib fat

The average rib fat across the seven producers was 15.6 ± 3.04 mm, cattle from Producer A general had higher rib fat coverage in comparison to the other producers (Table 19). Rib fat was significant in the model ($P < 0.001$) and had a positive influence on compliant pH (Table 1). As rib fat increased by 1 mm the odds of a carcass being classified as pH non-compliant was 0.844 times the odds of a carcass with a 1 mm reduction in rib fat depth. Higher means were seen in the pH <5.7 category's for both male and female carcasses (Figure 5). Any score of 30 mm rib fat or more was only captured under the pH pass category for females and males had no scores over 25 mm with a pH fail.

Table 19: The mean, median, minimum and maximum rib fat scores (mm) for each producer

Item	Producer						
	A	**B**	**C**	**D**	**E**	**F**	**G**
Mean	11.5 ± 4.5	7.43 ± 2.0	7.59 ± 2.5	9.08 ± 3.4	9.92 ± 3.7	6.22 ± 1.5	8.74 ± 3.6
Median	10.0	7.0	7.0	8.0	9.0	6.0	9.0
Minimum	3.0	1.0	2.0	1.0	1.0	2.0	1.0
Maximum	55.0	25.0	56.0	60.0	32.0	40.0	55.0

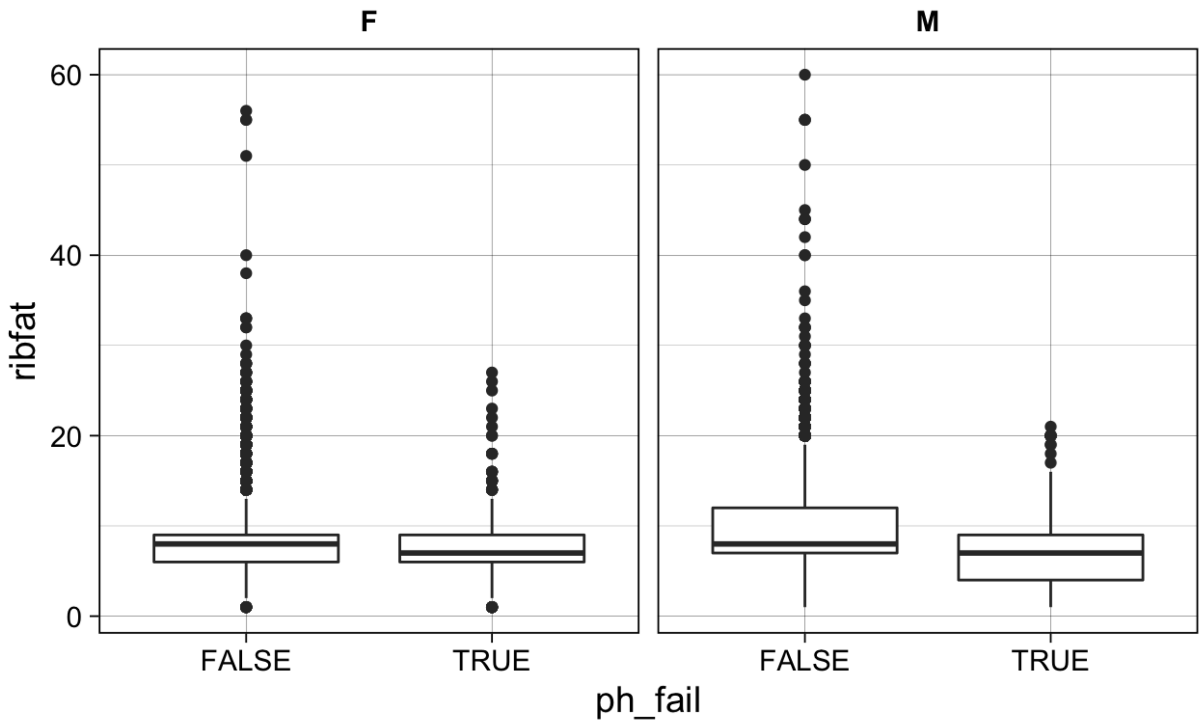

Figure 5: The mean, median, min and max rib fat mm for pH> 5.71 (True) or pH<5.70 (False) for males and females

4.6.5 Hump Height

The average hump height from producers was 61.3 ± 17.7 mm, although Producer G had the highest average hump height (Table 20). Hump height was significant in the model (*P* < 0.001; Table 1). As hump height increased by 10 mm the odds of a carcass being classified as pH non-compliant was 0.950 times the odds of a carcass with a hump measuring 10 mm less.

Table 20: The mean, median, minimum and maximum hump heights (mm) for each producer

Item	Producer						
	A	B	C	D	E	F	G
Mean	66 ± 18.1	49 ± 9.2	51 ± 12.2	64 ± 17.3	66 ± 14.9	47 ± 9.1	67 ± 19.9
Median	65.0	45.0	45.0	60.0	65.0	45.0	65.0
Minimum	15.0	20.0	20.0	15.0	30.0	20.0	20.0
Maximum	160	150	140	265	220	170	280

4.6.6 Fat Colour

Relative to fat colour 0, animals with higher fat colours were more likely to have high pH. Fat colour was significant in the model ($P < 0.001$; Table 1) with the odds of fat colour groups 1, 2, 3 and 4+ being 1.972, 2.465, 4.076 and 5.362 times more likely to be classified as pH non-compliant than the odds of fat colour group 0. This was reflected in the proportion of non-compliant carcasses for each fat colour group (Table 21).

Table 21: Total number of carcasses and percentage pH>5.70 for each fat colour group

Colour	Total Carcasses	Compliant	Non-Compliant	Proportion Non-Compliant
0	37 244	36 776	468	1.30 %
1	67 644	65 410	2 234	3.30 %
2	33 261	32 122	1 139	3.40 %
3	2 296	2 130	166	7.20 %
4 +	377	328	49	13.00 %

5 Discussion

5.1 Animal factors influencing dark cutting

5.1.1 Hormone Growth Promotants

Hormonal growth promotants have been under the suspicion of having adverse effects on carcass quality since they were first introduced (Grandin 1992). The use of HGP within this study were associated with an increased risk of pH carcass failures. However these findings are not consistent with the findings of (Steel *et al.* 2018). The authors reported that HGP use either no effect on the incidence of dark cutting or slightly reduced the incidence (Steel *et al.* 2018).

Previous studies have highlighted a relationship between HGP usage and an increased incidence of dark cutting (Morgan 1997; Scanga *et al.* 1998; Dikeman 2003; Hunter 2010). These authors also acknowledged that the degree of this affect depends on the type of HGP, the timing of its use, whether it was incorrectly implanted and if over-dosing occurred. Aggressive use of HGP (Morgan 1997) or false implant strategies can increase the susceptibility of cattle to stress, making them more prone to dark cutting when exposed to unusually stressful circumstances (Dikeman 2007).

There may also be an underlying effect of HGP on the susceptibility of different sexes to stresses imposed (Gaughan *et al.* 2005). Steers treated with an androgen HGP may exhibit an increase in aggressive behaviour as a result (Hunter 2010), which could increase the incidence of pH non-compliance due to a rise in stress and muscle contraction. This affect will be amplified if the steers are transported to slaughter while the HGP is still secreting significant amounts of the hormone or still within the 'pay-out period' (Hunter 2010). But the incidence of dark cutting may be decreased by slaughtering the cattle more than 100 days after being implanted with a HGP (Hunter 2010). Therefore the warnings and recommendations given by the manufacturers of HGP should be strictly adhered to in order to minimise the chance of adverse effects on the incidence of dark cutting.

5.1.2 Sex

Females had a higher incidence of pH non-compliance, when compared with steers in the raw data. When females are cycling, glycogen-depleting sexual activities such as mounting could account for this increased incidence in non-compliant MSA carcasses (Kenny and Tarrant 1988; Broom 2008), as this activity is escalated when females are in oestrus (Warren *et al.* 2010). The sex effect on the incidence of pH non-compliance may also result from differences in stresses experienced by females versus steers which may be driven by an animal's predisposition to stress as well as hormonal fluctuations. Studies have found that there is a significant association between gender and temperament (Voisinet *et al.* 1997b) and that heifers tend to be more excitable and more fearful than steers (Voisinet *et al.* 1997a) and therefore more susceptible to stress and dark cutting. From this raw data evidence, it seems logical that heifers need to be treated with greater care and all efforts should be made to minimise the pre-slaughter period and lairage time, reducing the opportunity for cycling animals to generate excitement, stress and physical activity which all impact on glycogen depletion.

When castrates are introduced into a group of females, increased physical activity resulting from this increase in mounting, chasing and excitement increases muscle contraction and therefore depletes muscle glycogen stores at a higher rate than if castrates and females had remained separate. This excitement could be the reason why males in this base model analysis had a higher odds ratio of being pH non-compliant. Our results are partially supported by the findings of Page *et al.* (2001) who found no difference in muscle pH between steers and heifers. However the findings from this year long feedlot survey have been contradicted by numerous other studies that also found steers to have lower incidences of dark cutting than females (Voisinet *et al.* 1997a; Wulf *et al.* 1997; Scanga *et al.* 1998; Warren *et al.* 2010; Romero *et al.* 2013).

5.1.3 Days on feed

Increasing days on feed increased was associated with a slight increase in incidence of pH non-compliance in this study. This could be due to older animals fibre type changes becoming more glycolytic (red type) and the term could be explaining further variation that ossification is not accounting for. Days on feed could also be significant as animals get older and fatter, their insulin sensitivity might drop but more importantly their adrenaline sensitivity increases, so they might burn more glycogen during the pre-slaughter period (Martin *et al.* 2011) showed this in sheep. As sheep aged they had larger response and increased glycogen utilisation (Martin *et al.* 2011).

The redness of meat has been shown to increase as myoglobin increases with animal age (Ledward and Shorthose 1971; Hopkins *et al.* 2007; Hughes *et al.* 2014). The results of this study show that graders at processors are biased in assuming high pH outcomes based on AUS-MEAT colour 4+, therefore we can assume that with increasing age, meat colour will appear darker and therefore the likelihood of pH fails in the dataset would also increase.

It is also possible that some of this variation could be explained by mixed lots. Poor performers and ill health cattle can be re-penned at the feedlot. These animals can be held back together as a new lot and slaughtered together when enough numbers are gathered. Therefore groups with an already increased likelihood of non-compliant MSA pH are held on feed longer before slaughter.

5.1.4 Feedlot pulls

Feedlot pulls were associated with an increased incidence of pH non-compliance in the current study. This was expected as pulled animals are exposed to increases in handling and external stimuli and therefore become more excitable, stressed and utilise more glycogen (Warriss 1990). If the pulled animal is identified as needing hospital treatment it will be drafted and moved to a hospital pen further increasing the size and duration of the stimuli. Hospital pens will usually hold cattle from mixed lots, they then establish social regrouping with each new introduction. The stress involved with regrouping when mixing cattle has been shown to directly impact muscle glycogen utilisation (McVeigh and Tarrant 1983). The underlying factor for feedlot pulls is why they were pulled in the first place. Sickness, injury and disease usually result in a reduction in DMI, it is well established in the literature that decreases in feed intake or fasting will increase the incidence of pH non-compliance in grain fed cattle, much like those on a lower plane of nutrition.

5.2 Weather factors influencing dark cutting

Climatic variables during the 7 days prior to feedlot exit were identified as risk factors for pH non-compliance within this study. Increasing average ambient temperature, rainfall, increasing average THI, increasing hours above HLI_{86} were associated with an increased incidence of pH non-compliance. Whereas increased average wind speeds and increasing solar radiation were associated with a decreasing incidence of pH non-compliance. This is not unexpected as wind speed is known well known to influence thermal exchange mechanisms (Esmay 1969; Gebremedhin 1985; Silanikove 2000). Furthermore, it has been established that the influence of heat load can be off set with an increase in air movement (Thompson 1974; Silanikove 2000; Berman 2005).

It is well established that periods of heat load are associated with a decrease in feed intake (Hahn 1985; Beede and Collier 1986; 2003; Brown-Brandl *et al.* 2005) and subsequently live weight gains (Mitlöhner *et al.* 2002). As ambient heat load increases, cattle divert energy that is typically partitioned for growth towards maintaining homeostasis (Kadzere *et al.* 2002; Ravagnolo and Misztal 2002), resulting in a reduction in growth and growth efficiency. Live weight gains during heat load periods are further confounded by increase in maintenance energy requirements of approximately 7 to 25 % (NRC 2001), which is associated with energy costs for dissipating accumulated heat load (Baumgard and Rhoads 2007), i.e. increased respiration rate. For feedlot cattle this diversion of energy is associated with depressed growth rates, whereby heat related decreases in weight gain are approximately 10 kg, which coincides with a seven day increase in days on feed (Baumgard and

Rhoads 2012). The reduction in feed intake, whole body exposure to stressors and redistribution of energy, may result in lower muscle glycogen thus increasing the odds of non-compliant MSA pH. However, further studies are required to examine the relationship between carcass attributes and climatic conditions in cattle. Furthermore, the influence of environmental conditions and/or time of exposure to these conditions on the incidence of non-compliant MSA pH is yet to be established.

5.3 Lairage factors influencing dark cutting

5.3.1 Transport time

Transport time did not increase the likelihood of pH non-compiance ($P = 0.355$), however this can be partly explained by there not being a large variation of transport times for each producer in the current study. This does align with work done by Ferguson *et al.* (2001) finding that transporting cattle < 400 km had no effect on dark cutting, only increasing the incidence slightly with distances over this (0.1-0.2 pH units). This was also found by Chulayo *et al.* (2016), suggesting cattle can acclimate during a 200-400 km transport distance but seem to have unidentified stress increases after that.

5.3.2 Time in Lairage

Increasing the time cattle spent in lairage was associated with an increase in pH non-compliance. This would be partly due to the effects of fasting cattle pre-slaughter throughout the time in lairage. This aligns with the work by Jones *et al.* (1986) showing that increasing fasting from 4-24h increased the pHu of meat in steers. Jones *et al.* (1990) showed that increases in feed and water withdrawal in cattle from 0 to 48h led to increases in pHu. Increasing the time spent in lairage would also effect the duration that lots of cattle are exposed to the processor lairage environment and the resultant stress. The period between feedlot and slaughter comprises of a multitude of stressors (Ferguson *et al.* 2001), so reducing this period will reduce the compounding impacts on muscle glycogen stores and ensure that glycogen concentrations will be as high as possible at slaughter. Time in lairage can be viewed as time in which stress and exercise can occur, reducing muscle glycogen concentration during that period.

5.3.3 Climatic conditions during lairage

During lairage there was no relationship between relative humidity, ambient temperature or solar radiation and odds of pH non-compliance. Increasing wind speed and rainfall increased odds of pH non-compliance. However it is difficult to define the influences of climatic conditions during lairage on pH non-compliance beyond these terms. Further data analysis is required to develop an understanding of the relationship between climatic conditions and pH non-compliance. Furthermore within study it is not yet apparent if the relationship between pH non-compliance and climatic conditions is associated with seasonal variability. Until knowledge regarding these factors is developed reducing time in lairage, as highlighted within this study, may reduce the incidence of pH non-compliance.

5.4 Processor factors influencing dark cutting

5.4.1 Time to grading

Time between slaughter and grading is required to allow glycogenolysis to occur, producing lactate and hydrogen ions, which subsequently cause muscle pH to decline from around neutral at the time of death to an ultimate level around 5.5. A limited pH decline results in a high pH (pH >5.7) and a dark colour at grading, as opposed to a full pH decline to 5.4–5.5, which results in a bright cherry-red colour at grading (Murray 1989). The time to grading results of the 12 month supply chain survey indicate that some carcasses are being graded before they reach ultimate pH and are falsely classified as pH non-compliant. The results from experiment 3 (B.FLT.0399) also suggest that carcasses are graded too early and that grading should occur after a minimum of 20 hours. The results from the supply chain survey independently support grading after 20 hours also.

5.5 Carcass factors influencing dark cutting

5.5.1 Hot standard carcass weight, marbling and rib fat

Findings from this study show that heavier carcasses, with higher MSA marbling scores and rib fat depths have lower incidences of pH non-compliance. These characteristics all pertain to an animal's mode of nutrition and metabolisable energy intake in the finishing phase and therefore complement the results relating to nutrition. Animals finished on higher energy intakes produce fatter carcasses in all depots, including intramuscular and subcutaneous depots or marbling and rib fat (Harrison *et al.* 1978). Furthermore it can be assumed that heavier carcasses at the same age as lighter carcasses are also the result of having better nutrition levels (McGilchrist *et al.* 2012). This is in alignment with the findings of Kreikemeier *et al.* (1998) who found a decrease in DFD incidence of 0.94% to 0.6% as the mean carcass weight of a slaughter group increased. Findings of McGilchrist *et al.* (2012) support our results for rib fat depth and carcass weight. Furthermore Page *et al.* (2001), found that for carcasses with fat thicknesses below approximately 7.6 mm, muscle ultimate pH values were higher and muscle colour appeared darker.

5.5.2 Fat colour

The fat colour effect on the incidence of pH non-compiance in this study is an interesting outcome. The fat colour of grass fed beef typically appears more yellowish than the whiter fat colour of grain fed beef due to having higher levels of β-carotenoid (Daley *et al.* 2010). The results of this study showed that carcasses with higher or yellower fat colour scores were shown to have a higher incidence of pH non-compliance which may be attributed to being in a state of ketosis or were in a state of ketosis prior to entering the feedlot. This result was evident even when DOF was included in the statistical model indicating that this finding is not driven by DOF alone. Even though there were no grass fed cattle in the data set, due to the variations in fat colour it is possible that these animals were either short days on feed or in a state of ketosis. However further investigations are required to understand the factors relating to higher, or increasing, fat colour scores in grain fed cattle and its relationship with non-compliant MSA pH. Walker *et al.* (1990) found that carcasses with extremely yellow fat predominantly occurred in older cattle that had been grass fed. This could be the result of the accumulation of β-carotenoid over time in response to seasonal nutritional variations, however the true relationship between fat colour, time on grain feed and muscle glycogen has had limited investigations. The effect of being in a state of ketosis on fat colour and the resultant effect on carcass pH is also not known. However the results from this study suggest that a strong relationship exists.

5.5.3 Ossification

Groups of animals with a higher average age and maturity compared to other groups have a higher incidence of pH non-compliance. This result aligns with the findings of (McGilchrist *et al.* 2012) who found that cattle that grow faster, as indicated by having lower ossification scores compared to other cattle of the same carcass weight, have lower incidences of non-compliant pH in beef. This is due to the fact that good nutrition increases animal growth rates and increases muscle glycogen concentrations (Gardner *et al.* 2001) which decreases the occurrence of non-compliant pH.

Another possible reason for this finding could be the fact that younger cattle indicated by lower ossification scores have a higher proportion of fast glycolytic type IIX muscle fibres than older cattle (Brandstetter *et al.* 1998). A higher proportion of these muscle fibres enhances their ability to synthesise muscle glycogen (McGilchrist *et al.* 2012). Therefore it is likely that younger cattle will have higher muscle glycogen content (McGilchrist *et al.* 2012). Wegner *et al.* (2000) also supports this concept as they demonstrated that paler coloured meat was related to cattle having a higher frequency of type IIX muscle fibres. Evidently, in order to minimise the cost of pH non-compliance, producers should sell cattle at younger ages or ensure that if older cattle are sold for slaughter they are appropriately managed in terms of nutrition in the weeks prior to slaughter so as to minimise the risk of failing on pH.

5.5.4 Hump height

Within the current study as the *Bos indicus* content of cattle increased, as measured by increased hump height, the incidence of pH non-compliance declined. This finding is supported by Lorenzen *et al.* (1993) reporting that the incidence of dark cutting were 4.7 % and 4.4 % for *Bos taurus* and *Bos indicus* carcasses respectively. The decreased incidence of dark cutting in *Bos indicus* cattle could be due to these cattle being less susceptible to stress than *Bos taurus* cattle as shown by Tyler *et al.* (1982). However, some studies have found no muscle colour differences between *Bos indicus* type carcasses and *Bos taurus* (Voisinet *et al.* 1997a; Page *et al.* 2001). This could be the result of relatively small numbers of cattle analysed and low proportions of *Bos indicus* cattle compared to *Bos taurus.* It is reasonable to conclude that *Bos indicus* cattle have lower incidences of pH non-compliant than *Bos taurus* due to their decreased susceptibility to stress and therefore a lower level of muscle glycogen depletion in the pre-slaughter period.

5.6 Achieving Project Objectives

The objectives of this study were to:

1. Conduct a 12 month slaughter chain audit of grain-fed cattle at a minimum of three processors with a known incidence of dark cutting, and their supplying feedlots, to determine factors contributing to variation in dark cutting.
2. Describe recommendations to minimise the incidence of dark cutting carcasses in Australia.

This study conducted 12 month intensive supply chain audit between September 1, 2017 and August 31, 2018. Three processing facilities (abattoirs) and seven producers (feedlots) were enrolled within this study. The incidence of dark cutting was variable between the abattoirs and feedlots, with the greatest incidence of dark cutting being 3.15 % and 3.26 % respectively. The major recommendations from this study are i) reduce the duration of time cattle spend in lairage and ii) increase time to carcass grading. A detailed list of recommendations are described below.

6 Conclusions/recommendations

6.1 Conclusions

- The factor with the largest odds ratios seen in the experiment was fat colour. All groups 1-4 had increasingly higher odds ratios compared to group 0, which is interesting for lot fed cattle on low β-carotene diets analysed with days on feed in the model. This indicates that cattle that have yellow fat at feedlot exit are a higher risk of pH non-compliance. The metabolism driving this risk remains to be elucidated but may be due to ketosis.
- Time to grade was significant in the model and consistently reduced the incidence of pH non-compliance, when grading occurred after 20 hours. Plants are likely grading too early for some carcases and the incidence of pH non-compliance would likely reduce if grading later or a re-grading strategy was implemented.
- Time to grade had a greater effect for reducing high colour than pH non-compliance. Splitting carcasses later will reduce the incidence of high colour if implemented into a re-grading strategy.
- Time in lairage was significant and increased the odds of failing on pH as lairage time was extended, suggesting that a potential management strategy may be to reduce the amount of time cattle spend in lairage. Time off feed was also significant however transport time was not significant in this current data set. Increasing wind and rain during lairage (day of arrival) also significantly increased the incidence of pH non-compliance.
- Climatic conditions at the feedlot was significant, although the effect overall on the odds ratios for high pH was small. Increasing heat load during the 7 days prior to feedlot exit were associated with small increases in non-compliant MSA pH incidence. This was reflected by increasing average temperature, increasing temperature humidity index and increasing duration of time above HLI_{86}. Increased average rainfall in the week before feedlot exit increased the likelihood of failing on pH, as did increasing average wind speed across the 7 days before feedlot exit also decreased the odds of pH non-compliance.
- Sex was significant in the base model and males were correlated to increased odds of pH non-compliance, however this was not reflected in the raw data with females having a higher incidence of pH non-compliance. This appeared to be confounded by feedlot, when Producer G, Producer A, Producer E and Producer D were removed from the model for the lairage analysis, and the correlation between male and high pH reversed with males being less likely to be classified as non-compliant for pH. This was also reflected for the time in lairage analysis which only included Producer F, Producer B and Producer C.
- Hormonal growth promotants had a large significant effect on the incidence of pH non-compliance in this data set with HGP treated cattle having more than two times the odds of failing on pH of a HGP free cattle.
- Animals that had been 'pulled' at the feedlot, plus those with longer days on feed had higher incidences of pH non-compliance.

- Carcass phenotype traits were also associative with the incidence of pH non-compliance. Lighter carcasses, with lower hump heights, less rib fat, lower MSA marbling scores, and higher ossification had higher incidences of pH non-compliance.

6.2 Recommendations

- Data management and integrity needs improvement across the entire supply chain. It needs to be easier for data captured on cattle at the producer and processor level in order to be merged and compiled to allow better performance tracking and feedback. Within the current system it would likely be very difficult to implement any management or infrastructure changes which could benefit production or meat quality. A centralised database capable of receiving data from multiple levels of the supply chain, verify the integrity of the data and integrate it in a way that stakeholders can produce meaningful reports and assess outcomes would be beneficial.
- Plant graders were significant in the base model (however were removed from the base model as there was too few data for some graders and they were all confounded with processor) and they really should not be significant for pH. Carcass data collection needs to be as objective and accurate as possible as it underpins the entire MSA system. Not correctly using equipment such as pH meters and temperature probes, grading pH based on colour alone or rounding off on measurements decreases the correlation between recorded data and biological norms.
- Using the pH meters will reduce false positive pH non-compliance. This will also allow us to analyse biological reliant variables. Conducting research on inaccurate pH and temperature results is not ideal when working with continuous variables. For this study it was manageable as we needed pH fail yes or no. However, incorrectly recorded data also does not allow for accurate analysis of the biological impacts of traits like temperature at grading or the time to grading by temperature at grading relationship.
- While the impact of weather was fairly small in this data set, there is still a significant effect of high temperatures on the incidence of pH non-compliance. With climatic conditions changing and areas of Australia becoming hotter each year, heat load mitigation opportunities are certainly something that will benefit producers in the long run. Weather events cannot be altered however our management of cattle prior to and during the pre-slaughter period can be manipulated. Cattle that have experienced a climatic event which compromises their glycogen concentration should be managed carefully through the pre-slaughter period. This can be achieved to the greatest extent via reducing time in lairage.
- Rain and wind during the day of receival appears to heighten the percentage of pH non-compliance dramatically, possibly due to increased levels of stress. This means that cattle that arrive on rainy or windy days should have minimal time in lairage as possible. Two hours would be optimum.
- Pulled animals or lots with high pull rates, females and cattle that have received HGPs should also be identified and handled with extreme care as they are at greater risk of pH non-compliance.
- Increasing fat colour increased pH non-compliance. This was an unexpected outcome due to the data all coming from grain fed cattle. Research into what is causing the increasing fat colour scores in grain fed cattle may uncover a mechanism affecting the incidence of pH non-compliance, however it is likely linked to animals that are losing weight.

- Longer times between kill and grading corresponded to a decrease in the incidence of pH non-compliance in this data set. Carcasses graded during the 24-48 hour post slaughter window had the lowest odds of non-compliant MSA pH and high colour, however grading at a minimum 20 hours is ideal to give carcasses every opportunity to have a pH measurement below 5.7 and AUSMEAT meat colour <4. This will reduce the amount of false positives for failure on pH and minimise income losses to producers and processors. If processors are unable to grade after 20 hours due to commercial constraints, a re-grading strategy should be developed for carcasses that fail. Processors have a responsibility to ensure producers are not penalised unfairly for carcasses that would have been compliant if given the opportunity.
- Greater time in lairage was significantly correlated to an increase in pH non-compliance. Management across the supply chain should aim to have cattle in lairage for as short a time as possible as this will reduce the incidence of dark cutters. Same day kills would be ideal (following 2 hours in lairage to cool down and re-hydrate) however the ante mortem inspectors need to be available throughout the day to assess arriving cattle. If this is not possible then this issue should be addressed with AQIS by RMAC.
- At both processors where intensive lairage observations occurred animal management from arrival to slaughter was observed to be of a high standard. There did not appear to be any animal welfare and/or stock handling concerns. This standard needs to be maintained through appropriate training for new staff members. Processor A and Processor C had minimal handling of arriving lots of cattle and had zero pen movements as standard during the lairage period. Plant B had one additional pen movement on kill day for *ante mortem* inspections however the inspection pens were directly opposite the holding pen. Plant A would only have an additional pen movement when arriving cattle were very dirty, they would be placed into an initial pen with ground sprinklers on arrival for around 10 minutes before being moved to holding pen.
- Shade was adequate at Plants B and C however around half the pens were shaded at plant A. While not enough uncompromised data was gathered data to make sound recommendations, anecdotally cattle acclimatised sooner when placed in shaded pens on hot days than in full sun.
- Water availability and the animals likelihood of drinking was highlighted as a concern at Processor A. Further studies should be conducted to determine if dehydration of cattle at Plant A is causative for pH non-compliance.

7 Key messages

This study has generated a greater understanding of the factors within the supply chain that influence the incidence of dark cutting in grain-fed beef. Developing a greater understanding of the factors increasing the risk of dark cutting will allow for the development of effective management strategies to reduce the incidence of dark cutting in the feedlot cattle.

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9 Appendix 1

9.1 Model exclusions

9.1.1 Feedlot cattle weights

Cattle live weight from feedlot entry and exit became non-significant once days on feed (**DOF**) and hot standard carcass weights (**HSCW**) were included in the model.

9.1.2 Processor pen size and shade allocation

Pen size and shade allocation was confounded by processor so was not included in the analysis. The stocking density of lairage pens were 2.40 m^2/head and 3.96 m^2/head at Plant A (8 m × 6 m) and Plant B (9 m × 22 m) respectively.

Shade was provided to 50 % of the lairage pens at Plant A. Lots of cattle were segregated across multiple pens, thus confounding these data as individual animals from the same lot were housed within multiple pens. With no process for identifying which individuals were in which pens they could not be distinguished within lot. At Plant B had all pens were shaded except for inspection pens which were the same size directly across from home pens. Cattle were moved across to the inspection pens at 0400 h on kill day allowing for veterinary inspection of cattle to occur prior to slaughter.

The variation in pH non-compliance due to pen density or shade in lairage will be accounted for within the processor term. If shade and pen size are actually needed to be analysed, then lots of cattle going through multiple plants with pen size and shade variation is needed.

9.1.3 Panting Scores

Panting score observations have not been included in the dataset due to there not being enough hot days with panting scores to have statistical significance. Cattle were assessed for whether they exhibited panting on arrival yes/no and then a panting score of 1-5 one hour after arriving to home pen in lairage. As it was percentage of lot based observations it was confounded as lots were spread between pens with some shaded and with some having access to water and some not. Which individual animals were in the non-shaded pens could not be determined.

Panting scores were only ever seen during January and March at Plant C, while Plant A had panting scores during January through to April. There were 21 lots at Plant A and 8 lots at Plant C recorded during these periods. At Plant A, 71% of the lots recorded during this period had a Panting score of at least 1, an hour after arriving at home pen in lairage. 30% of these lots of cattle had a panting score of 2 or more. At Plant C 75% of lots during this period had a panting score of at least one however there was only one panting score of 2 recorded. The highest panting score seen was 3 at plant A on the 7/01/2018 which arrived at 2.00pm into lairage.

Observationally at Plant A, the proportion of cattle in the same lot without shade had a slower reduction in panting than the proportion that had shaded home pens. The cattle that could access water at Plant A had lower individual scores than those that did not have access to water.

While there are not enough numbers of lots for a statistical significance on the effect of dark cutting, observationally cattle with access to shade and water when arriving into lairage fared much better in the heat than those that did not.

9.1.4 Pen movement at processors

The number of pen movements was confounded between processing facilities. Plant A and Plant C did not move cattle between pen movements until cattle were being prepared for slaughter. Whereas, Plant B had a home pen for day of arrival until the following morning where all cattle were moved into pens for veterinary inspection as previously described. Cattle then remained within these pens until being marshalled for slaughter.

9.1.5 Number of washes at processors

The number of washes were confounded within processors as all three plants used the same process each day. Plant A conducted 2 washes, i) an automatic underfloor sprinkler system, and ii) via manual hand held hose prior to entering the knocking box. At times and extra wash could be conducted if cattle were classified as 'very muddy' on plant entry, however these were only noted during the intensive visits and were too few observations to conduct statistical analysis. Similarly, Plant B conducted 2 washes, i) an automatic underfloor sprinkler system, and ii) via manual hand held hose prior to entering the knocking box.

9.1.6 Influence of grader

Grader was not incorporated into the base model, even though there are significant differences between graders. Incorporating graders confounds the ability to identify plant level differences, which are more important, thus graders are nested within plants. This was due to some graders having very few numbers of carcasses graded, in addition the same method and equipment used to assess each carcass. The pattern of pH measurements was not consistent across graders (Figure 6; Figure 7; Figure 8) There was also an extremely strong unrealistic relationship between pH and meat colour at Plant B and Plant C, specifically there were no carcasses defined as non-compliant due to high pH on meat colour scores of ≤ 3. Additionally there were no compliant carcasses, based on pH, where meat colour was ≥ 4, from graders 5 and 13 at plant B (Figure 7). Similarly, Plant C had no compliant carcasses with meat colour scores of ≥ 4 (Figure 8). Plant A had the most realistic pH grading data with pH fails above and below meat colour score 4 (Figure 6).

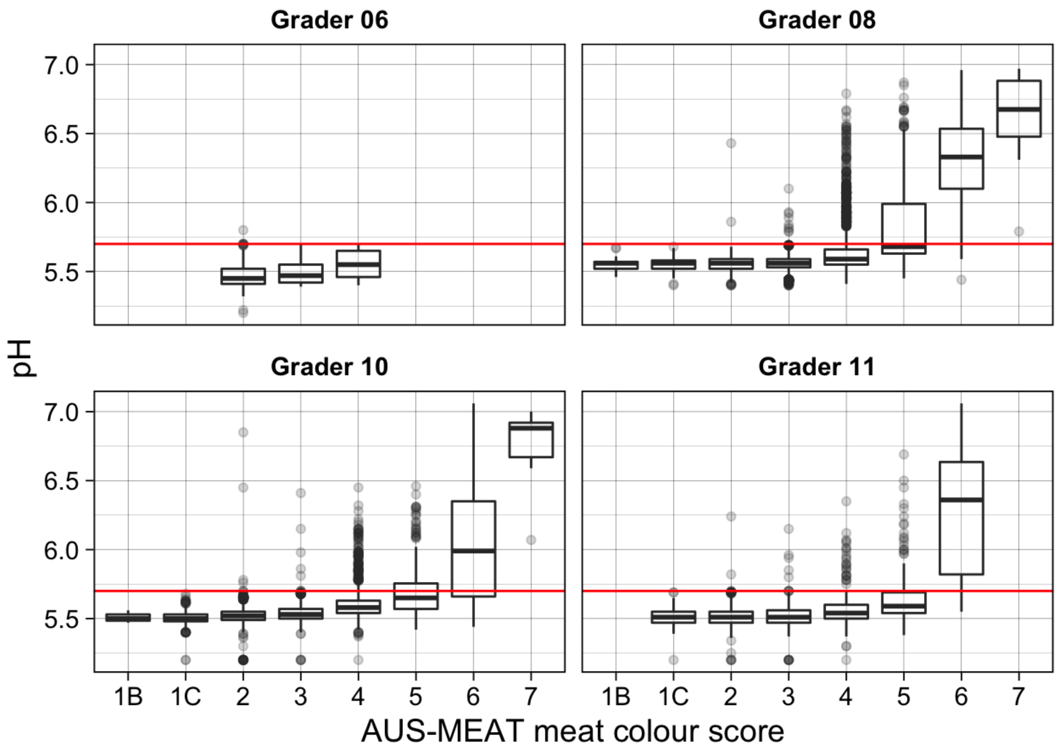

Figure 6: Plant A graders ph versus meat colour

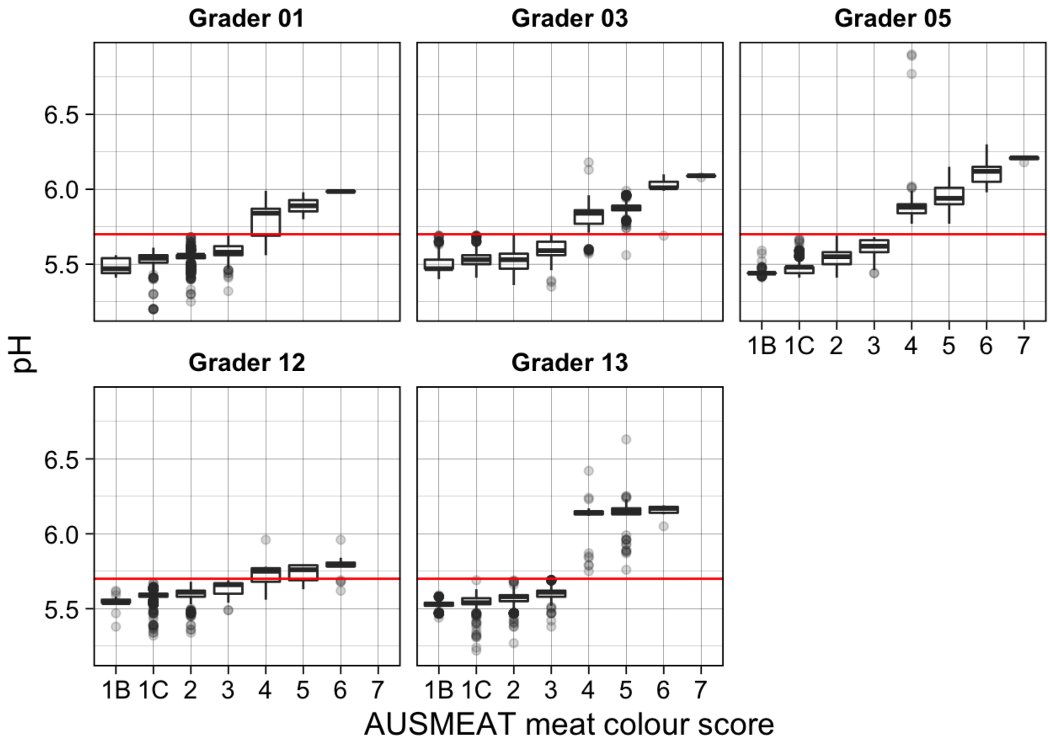

Figure 7: Plant B Graders pH versus meat colour

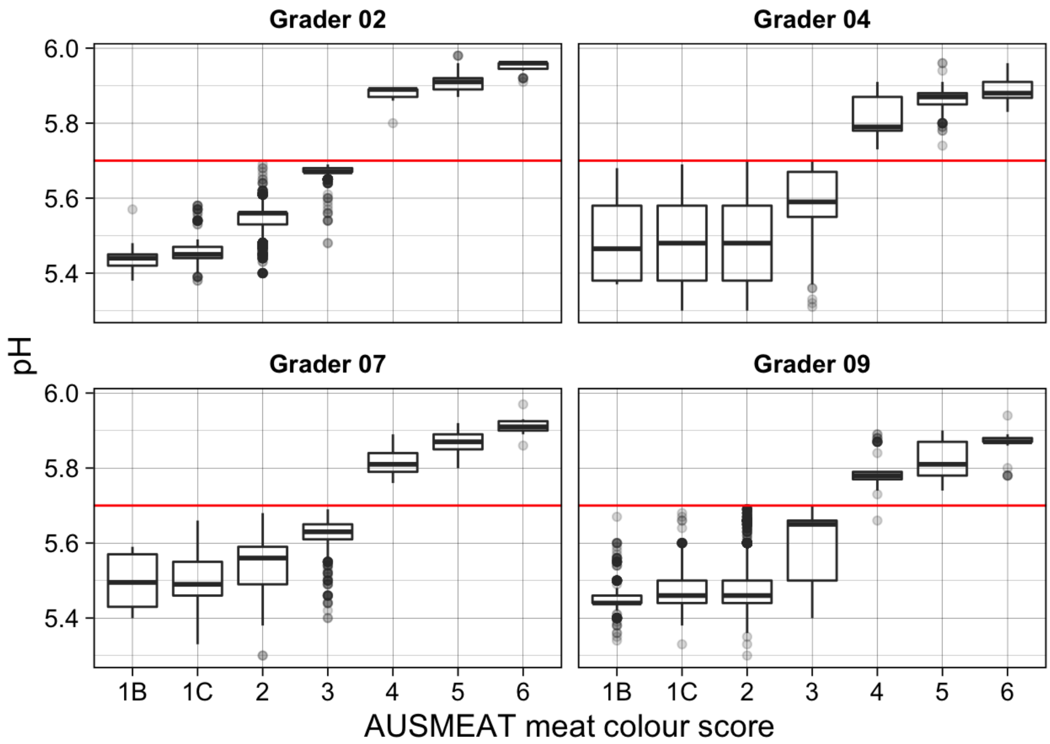

Figure 8. **Plant C graders pH versus meat colour**

When pH was visualised with marbling, grader 13 from Plant B has almost no pH recordings at all between a pH of 5.7 and a pH of 6.2 (Figure 9), these gaps of no recorded data were also observed in grader 2 and grader 7 from Plant C 7 (Figure 10). This is not a reflection of true pH decline in carcasses.

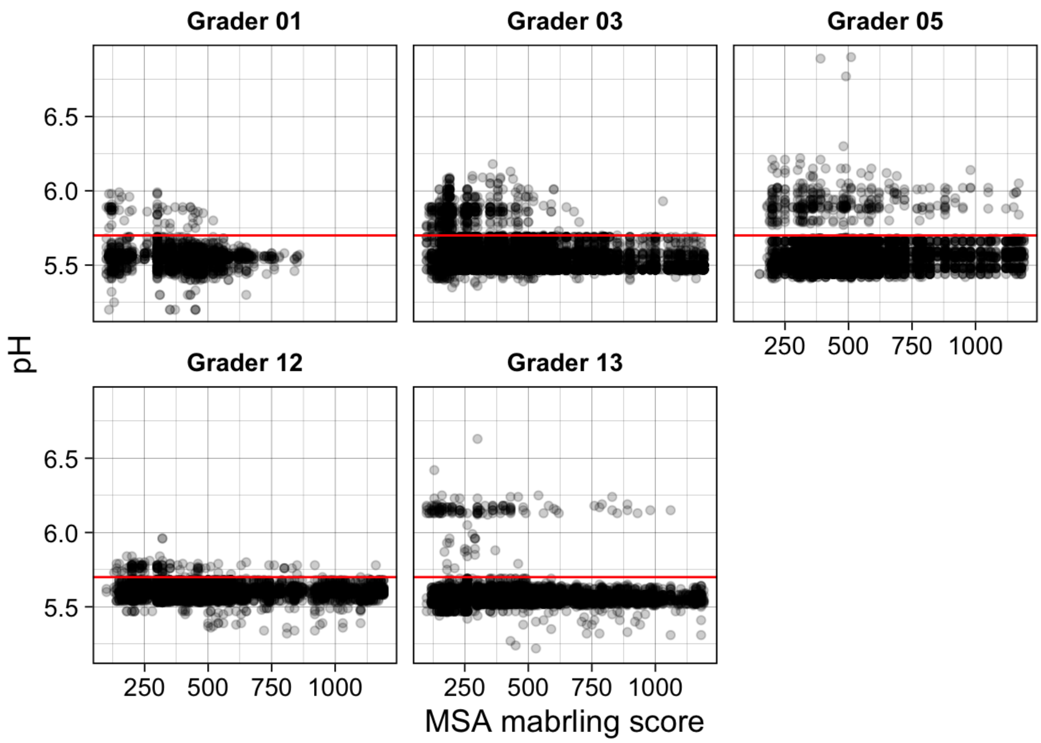

Figure 9: Plant B pH versus Marbling

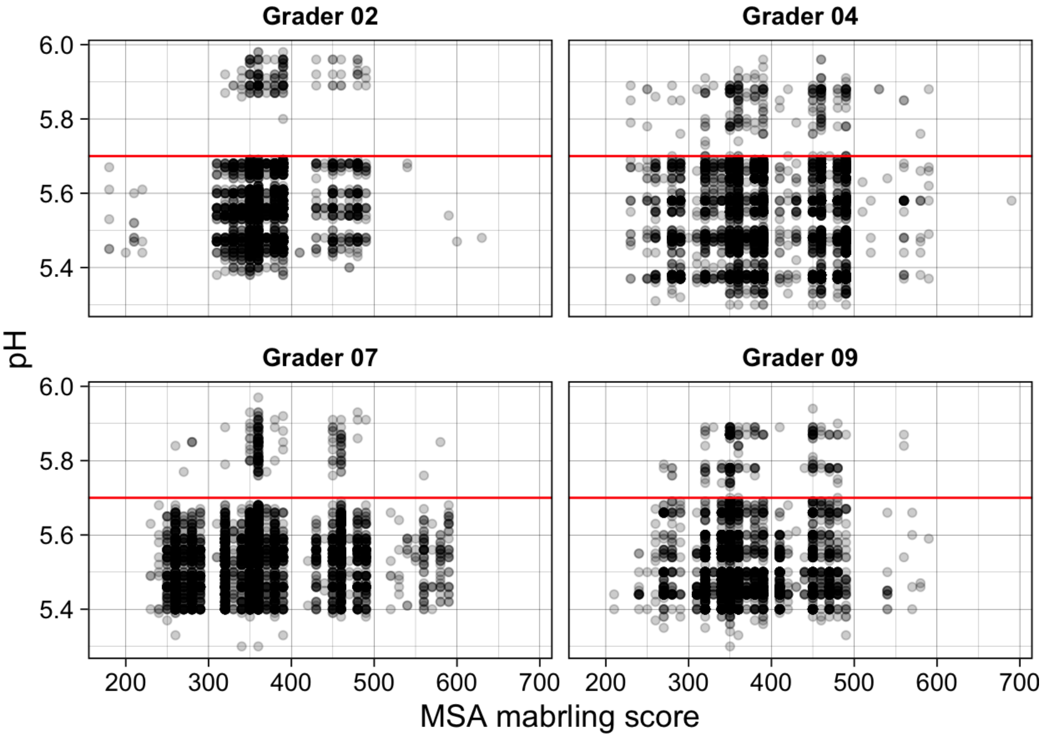

Figure 10: Plant C pH v Marbling

Loin temp was also not consistent across graders, for Plant C grader 9 appears to only record temps at 4 °C, 5 °C, 6 °C, 7 °C, 8 °C and 9 °C (Figure 11). This is a recorded estimation and not a reflection of true temperature.

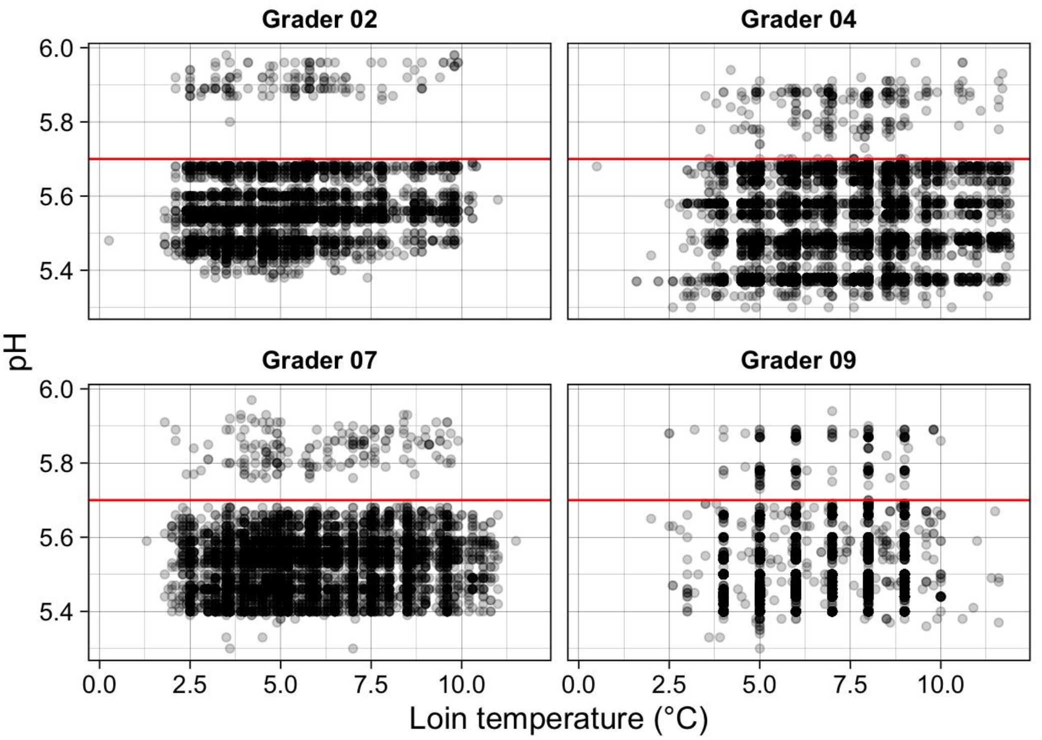

Figure 11: Plant C pH versus Loin Temperature

10 Appendix 2: High colour analysis

10.1 Base Model

The baseline model (Table 1), with an indicator variable high meat colour as the dependent variable. Variables were fit as generalised linear models with a logistic link function, such that the estimated coefficients may be interpreted as log-odds (or odds ratios when exponentiated). Where appropriate, plant and producer were always included as main effects. Most variables showed up as statistically significant, but this is in large associated with the sample size.

Table 1: Base model for high meat colour odds ratio prediction including significant variables

	High meat colour		
Predictors	*Odds Ratios*	*Confidence Interval*	*p*
(Intercept)	5.5303	4.3374 – 7.0511	**<0.001**
dof 10	1.0396	1.0322 – 1.0470	**<0.001**
hscw 10	0.9046	0.8992 – 0.9101	**<0.001**
hgpY	1.4383	1.3390 – 1.5449	**<0.001**
fc_grp1	2.5632	2.4142 – 2.7214	**<0.001**
fc_grp2	2.3686	2.2253 – 2.5211	**<0.001**
fc_grp3	3.7399	3.2749 – 4.2709	**<0.001**
fc_grp4+	5.0897	3.8124 – 6.7950	**<0.001**
tt_grp12-16 hrs	0.5401	0.5035 – 0.5793	**<0.001**
tt_grp16-20 hrs	0.6573	0.5865 – 0.7367	**<0.001**
tt_grp20-24 hrs	0.5310	0.4626 – 0.6095	**<0.001**
tt_grp24-48 hrs	0.2991	0.2354 – 0.3799	**<0.001**
tt_grp48+ hrs	0.2025	0.1822 – 0.2251	**<0.001**
hump 10	0.9458	0.9338 – 0.9580	**<0.001**
ribfat	0.8636	0.8569 – 0.8703	**<0.001**
mb_grp300-500	0.5059	0.4745 – 0.5395	**<0.001**

mb_grp500-700	0.6609	0.5678 – 0.7693	**<0.001**
mb_grp700+	0.4047	0.3007 – 0.5448	**<0.001**
oss 10	1.0634	1.0561 – 1.0708	**<0.001**
M	0.9638	0.9053 – 1.0261	0.249
Plant B	0.2213	0.1706 – 0.2871	**<0.001**
Plant C	0.0581	0.0414 – 0.0815	**<0.001**
Producer B	1.4422	1.0214 – 2.0362	**0.037**
Producer C	1.0610	0.7821 – 1.4392	0.704
Producer D	1.1778	1.0756 – 1.2897	**<0.001**
Producer F	0.8055	0.5575 – 1.1639	0.249
Producer G	1.1554	0.9225 – 1.4472	0.209
Observations	140822		
Tjur's R^2	0.138		

Incidence of High Colour

10.1.1 Incidence by processor

Plant A, Plant B and Plant C had a high colour incidence of 13.4 %, 3.38 % and 2.56 % over the duration of the study (Table 2). Plant was a significant variable in the base model and the odds of carcases being classified high colour at Plant B and C were 0.22 and 0.058 times the odds of high colour occurring at Plant A (*P* < 0.001; Table 1). There were no differences in likelihood of different odds of high colour occurring at Plant C in comparison to Plant A (*P* = 0.704).

Table 2: Number of carcasses classified as normal (Colour < 4) and high colour (Colour > 4) across the three processing facilities

Plant	Total Carcasses	Normal Colour	High Colour	Proportion High Colour
A	68 431	59 235	9 196	13.4 %
B	41 693	40283	1410	3.38 %
C	30 698	29912	786	2.56 %

10.1.2 Incidence by feedlot

Producer D supplied the most cattle with 62 349 head, followed by Producer G (19,147 head), Producer C (18,989 head) and Producer A (18,546 head), respectively (Table 3). Producer D (13.7 %) had the highest incidence of high colour followed by Producer E (10.3 %) and Producer G (5.67 %;

Table 3), whereas the lowest incidence of non-compliance was observed from Producer A (1.37 %; Table 3).

Table 3: Number of carcasses classified as normal (Colour < 4) and high colour (Colour > 4) across the seven producers

Producer	Total Carcasses	Normal Colour	High Colour	Proportion High Colour
A	18 546	18291	255	1.37 %
B	7 472	7235	237	3.17 %
C	18 989	18506	483	2.54 %
D	62 349	53780	8569	13.7 %
E	6 082	5455	627	10.3 %
F	8 237	8102	135	1.64 %
G	19 147	18061	1086	5.67 %

The influence of producer on high colour was significant within the base model (Table 1). Cattle supplied by Producer B were 1.44 times the odds for being high in colour when compared to cattle supplied from Producer A ($P < 0.05$). Cattle supplied from Producer D were 1.17 times the odds of having high colour than the odds of high colour from Producer A ($P = 0.001$). The odds of carcasses being classified as high colour from Producer C ($P = 0.704$), Producer F ($P = 0.249$) or Producer G ($P = 0.209$) were not different from Producer A.

10.2 Animal Factors influencing high colour

10.2.1 Influence of hormone growth promotants

Hormonal growth promotants (HGP) were implanted in 72.7 % of the cattle captured within this study (Table 4). Producer B and Producer F had a HGP usage rate of 100 %, whereas Producer A did not use HGP in their cattle (Table 4), hence for the analysis, these three producers and HGP status are confounded. The cattle implanted with HGP had high colour incidence of 9.74%, whereas HGP free cattle had an incidence of 3.70 % (Table 5). Cattle implanted with HGP were 2.29 times the odds for being non-compliant based on high marbling when compared to cattle that were HGP free ($P < 0.001$; Table 1), which is confounded with producer so impossible to attribute to HGP status.

Table 4: Number of cattle implanted with Hormone Growth Promotants (HGP; %) across the seven producers during the study

HGP	Producer							Overall HGP
	A	B	C	D	E	F	G	
Yes	0 (0 %)	7 472 (100 %)	15 899 (83.7 %)	55 129 (88.4 %)	5 741 (94.4 %)	8 237 (100 %)	9 933 (51.9 %)	72.7 %
No	18 546 (100 %)	0 (0 %)	3 090 (16.3 %)	7 220 (11.6 %)	341 (5.6 %)	0 (0 %)	9 214 (48.1 %)	27.3 %

Table 5: Number of carcasses classified as normal (Colour < 4) and high colour (Colour ≥ 4) as evaluated by hormone growth promotant

HGP	Total Carcasses	Normal Colour	High Colour	Proportion High Colour
No	38 411	36 989	1422	3.70 %
Yes	102 411	92 441	9970	9.74 %

10.2.2 Influence of sex

Males accounted for 71.5% of cattle within the study (Table 6). Producer B fed a higher proportion of female cattle (96.6 %), whereas Producer G (92.7 %) and Producer E (99.9 %) fed a higher proportion of male cattle (Table 6). Female and male carcasses had a total high colour incidence of 6.58 % and 8.68 %, respectively (Table 7). Sex was not significant in the model (P= 0.249).

Table 6: Distribution of female and male cattle fed across the seven producers during the study

Sex	Producer							Overall
	A	B	C	D	E	F	G	
Female	2 488 (13.4 %)	7 221 (96.6 %)	14166 (74.6%)	10105 (16.2%)	6 (0.1%)	4756 (57.7%)	1401 (7.3%)	28.5 %
Male	16 058 (86.6 %)	251 (3.4 %)	4823 (25.4%)	52244 (83.8%)	6076 (99.9%)	3481 (42.3%)	17746 (92.7%)	71.5 %

Table 7: Number of female and male carcasses classified as normal (Colour < 4) and high colour (Colour > 4)

Sex	Proportion High Colour
Female	6.58 %
Male	8.69 %

10.2.3 Days on feed

Mean days on feed (DOF) across producers was 128 ± 76. Producer A had the highest days on feed with an average of 285 ± 92 days (Table 8). Producer F had the lowest average days on feed with 61 ± 3 (Table 8). Days on feed had a significant interaction in the model ($P < 0.001$). Increasing DOF was associated with a slightly higher incidence of the carcass being high colour. A 10 day increased in DOF increased odds of high colour by 1.0396 times (Table 1).

Table 8: The mean, median, minimum and maximum days on feed for cattle from each producer

Item	Producer						
	A	B	C	D	E	F	G
Mean	285 ± 92	82 ± 17	96 ± 36	105 ± 25	98 ± 7	61 ± 3	136 ± 38
Median	223	82.0	83.0	103	100	60.0	134
Minimum	22.0	8.0	43.0	8.0	70.0	60.0	69.0
Maximum	565	279	256	282	100	70.0	440

10.2.4 Feedlot morbidity

Feedlot morbidity was significant when included in the model (*P* < 0.001) and were associated with an increased likelihood in being classified as high colour (Table 9). If an animal was identified as morbid the odds of having a high colour at grading was 1.3308 times that of a healthy animal.

Table 9: Base model for odds ratio of high meat colour using feedlot morbidity

Predictors	Odds Ratio	Confidence Interval	Significance
Intercept	0.1783	0.1192 – 0.2668	*P* < 0.001
Morbid	1.3308	1.2576 – 1.4082	**<0.001**

10.3 Weather factors influencing high colour

10.3.1 Climate Model 1. Solar radiation, wind speed, rain, relative humidity and ambient temperature

Solar radiation max, wind speed, rain and relative humidity influenced the odds of carcasses being classified with high colour (Table 10). There was no relationship between average solar radiation (P =0.305), relative humidity max (*P* =0.112) and ambient temperature (average, P = 0.455; range, *P* =0.239; minimum, *P* =0.847; or maximum, *P* =0.192) and odds of high colour within this model Table 10).

Cattle exposed to a higher max solar radiation during the 7 days prior to feedlot exit had a slightly lower likelihood of being classified with high colour, when compared to animals that were exposed to lower solar loads (*P* < 0.05). The odds ratio for high colour for max solar radiation was 0.991 times the odds of having high colour if exposed to one average W/m^2 over the 7 days prior to feedlot exit. Similarly, higher average wind speeds over the 7 days prior to feedlot exit were associated with lower odds of being classified high colour (*P* <0.001). Cattle exposed to an average wind speed 1 m/s faster over the 7 days had 0.9250 times the odds of high colour, compared with an animal with an average wind speed 1 m/s slower. An increased rainfall during the 7 days prior to feedlot exit increased the odds of high colour ($P < 0.001$), where cattle that experienced 1 mm of rain more had 1.0135 times the odds of high colour when compared to cattle that were exposed to 1 mm less rainfall within the same period. Furthermore higher relative humidity during the 7 day prior to feedlot exit correlated with an increase in the odds of high meat colour ($P < 0.01$). Cattle that were exposed to an increased humidity by 1 unit had 1.0119 times the odds of high colour compared with cattle exposed to average relative humidity that were 1 unit lower during the 7 days prior to feedlot exit.

Table 10: The odds ratios for the effect of temperature (mean, range, max and min), solar radiation, wind speed, relative humidity and rain for the 7 days prior to feedlot exit on the incidence of high colour

Predictors	Mean Model		Range Model		Max Model		Min Model	
	Odds Ratio	**Significance**	**Odds Ratio**	**Significance**	**Odds Ratio**	**Significance**	**Odds Ratio**	**Significance**
Intercept	0.0147	**<0.001**	0.0828	**<0.001**	0.1177	**0.002**	0.0223	**<0.001**
SR_{MEAN}	0.9989	0.305	0.9987	0.104			0.9982	0.061
SR_{MAX}					0.9991	**0.019**		
WS_{MEAN}	0.9250	**<0.001**	0.9477	**<0.001**				
WS_{MAX}					0.9804	**0.003**		
WS_{MIN}							0.9604	0.825
RH_{MEAN}	1.0119	**0.006**						
RH_{RANGE}			0.9913	**0.049**				
RH_{MAX}					0.9923	0.112		
RH_{MIN}							1.0150	**0.002**
$T_{A, MEAN}$	1.0101	0.455						
$T_{A, RANGE}$			0.9846	0.239				
$T_{A, MAX}$					0.9986	0.912		
$T_{A, MIN}$							1.0021	0.847
Rain	1.0135	**<0.001**	1.0155	**<0.001**	1.0158	**<0.001**	1.0176	**<0.001**

10.3.2 Climate Model 2. Solar radiation, wind speed, rain, humidity and temperature humidity index

Solar radiation max, relative humidity, wind speed and rainfall influenced the odds of carcasses being classed high colour (Table 11). There was no relationship between solar radiation mean (P = 0.170), THI (mean, P = 0.166; range, *P* = 0.666; minimum, *P* = 0.263, or maximum, *P* = 0.113) and high colour in this model.

An increased in solar radiation max during the 7 days prior to feedlot exit was associated with an increase in the odds of carcases with high colour. SR max had 0.9987 times the odds of having high colour when compared to cattle that were exposed to conditions with a SR max 1 W/m^2lower ($P < 0.05$). An elevated relative humidity mean during the 7 days prior to feedlot exit was associated with increasing the odds of carcasses having a high colour. The odds ratio for carcases being classified as high colour were 1.0116 times the odds of cattle that had a RH mean of one unit less during the 7 days before exit (P<0.01). RH range had an inverse effect with the odds of having high colour having 0.9897 times the odds of an animal that had one RH range unit less in the 7 days before exit (P<0.05). Faster average wind speeds over the 7 day period were associated with lower odds of high colour. Cattle exposed to average wind speeds 1 m/s had 0.9246 times the odds of having high colour carcase when compared to an animal that was exposed to average wind speeds 1 m/s slower (P<0.001). An increase in rainfall was associated with an increase odds of high colour. Cattle exposed to 1 mm higher rainfall had 1.0136 times the odds of high colour than cattle exposed to 1 mm less rainfall during the 7 days prior to feedlot exit ($P < 0.001$).

Table 11: The odds ratios for the effect of THI (mean, max, range and min) plus solar radiation, wind speed, relative humidity and rain for the 7 days prior to feedlot exit on the incidence of high colour.

Predictors	Mean Model		Range Model		Max Model		Min Model	
	Odds Ratio	Significance	Odds Ratio	Significance	Odds Ratio	Significance	Odds Ratio	Significance
Intercept	0.0086	**<0.001**	0.0749	**<0.001**	0.0394	**<0.001**	0.0193	**<0.001**
SR_{MEAN}	0.9986	0.170	0.9986	0.078			0.9979	**0.035**
SR_{MAX}					0.9987	**<0.001**		
WS_{MEAN}	0.9246	**<0.001**	0.9505	**<0.001**				
WS_{MAX}					0.9799	**0.002**		
WS_{MIN}							0.6497	*P* = 0.038
RH_{MEAN}	1.0116	**0.007**						
RH_{RANGE}			0.9897	**0.014**				
RH_{MAX}					0.9928	0.121		
RH_{MIN}							0.9928	0.121
THI_{MEAN}	1.0131	0.166						
THI_{RANGE}			0.9964	0.696				
THI_{MAX}					1.0179	0.125		
THI_{MIN}							1.0043	0.524
Rain	1.0136	**<0.001**	1.0157	**<0.001**	1.0150	**<0.001**	1.0176	**<0.001**

10.3.3 Climate Model 3. Rain and heat load index

An increase in the number of hours above HLI_{86} during the 7 days prior to feedlot exit was associated with an increased odds of a carcass having high colour (Table 12). Cattle that were exposed to 1 h longer per day of HLI_{86} over the 7 day period were 1.0145 times the odds of having a high colour when compared to cattle that were exposed to HLI_{86} for 1 h less during the 7 days prior to feedlot exit. Interestingly average (P =0.092), maximum (P =0.750) and minimum (P =0.310) HLI were not associated with an increased likelihood of high colour. Additionally, within this model rain did not influence the likelihood of high colour ($P > 0.05$).

Table 12: The odds ratios for the effect of HLI mean ,max , min, $HLI_{<70}$[1] and HLI_{86}[2] plus rain for the 7 days prior to feedlot exit on the incidence of high colour

Predictors	Mean Model		Max Model		HLI_{86} Model		$HLI_{<70}$ Model	
	Odds Ratio	Significance	Odds Ratio	Significance	Odds Ratio	Significance	Odds Ratio	Significance
Intercept	0.0061	**<0.001**	0.0132	**<0.001**	0.0136	**<0.001**	0.0137	**<0.001**
HLI_{MEAN}	1.0155	0.092						
HLI_{MAX}			1.0020	0.750				
$HLI_{<70}$[1]							2.9433	0.310
HLI_{86}[2]					1.0145	**0.001**		
Rain	1.0023	0.545	1.0027	0.481	1.0025	0.508	1.0185	0.401

[1]number of days where HLI did not go below 70 for equal to or greater than 6 hours during the 7 days prior to feedlot exit

[2]number of days where HLI was > 86 during the 7 days prior to feedlot exit

10.4 Lairage factors influencing high colour

10.4.1 Time in lairage

Transport time was identified as not significant (*P* =0.856). This can be partly explained by there not being a very large range of transport times within each producer. Specifically, all of the transport times for Producer B (≈ 7 to 8 h), D (≈ 2 to 4 h), E (≈ 6 h) and Producer F (≈ 4 h) were similar for each lot of cattle (Figure 2). Producer C supplied Plants B and C and had a greater range of transport times with majority at 2 H.

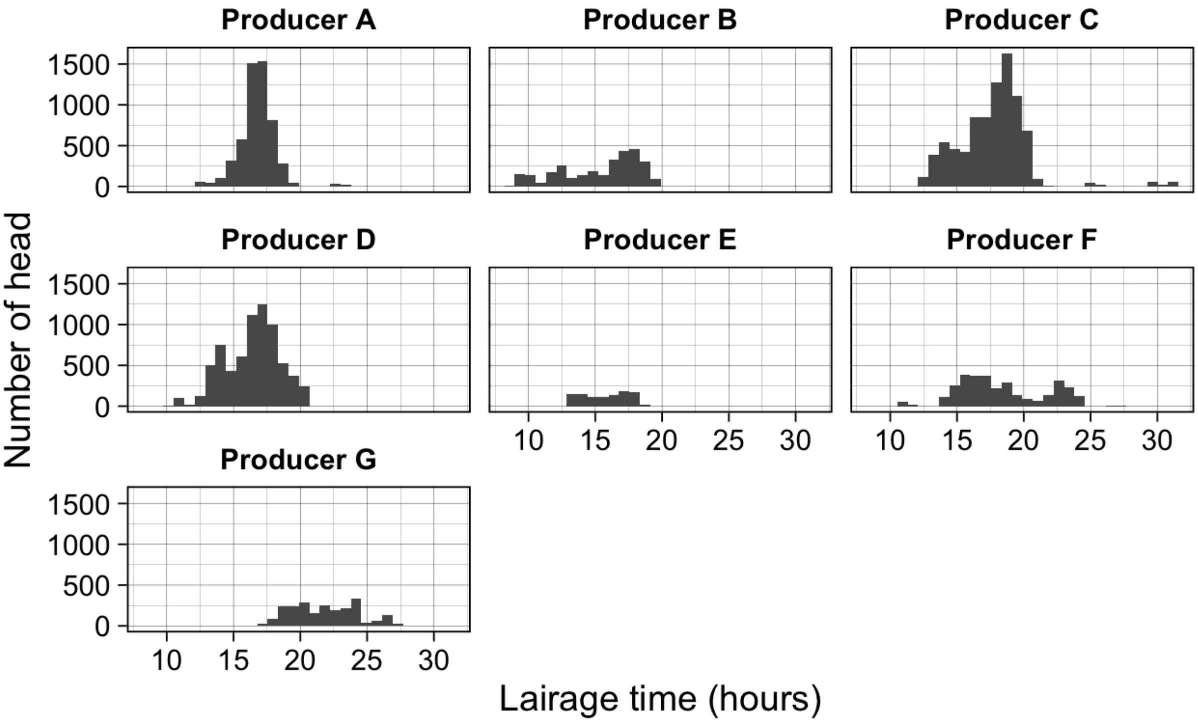

Figure 2: Approximate lairage duration(hours) for all producers

Time in lairage had a greater variability (Figure 3), and was associated to high colour scores ($P < 0.001$). The odds of high colour for animals who have an additional hour in lairage are 1.05 times the odds of animals without the additional hour of lairage (Table 13). Additionally transport time and time in lairage were used to time to estimate time off feed. Time cattle were off feed was also associated with an increased high colour ($P < 0.001$) and had an odds ratio of 1.05 (Table 13).

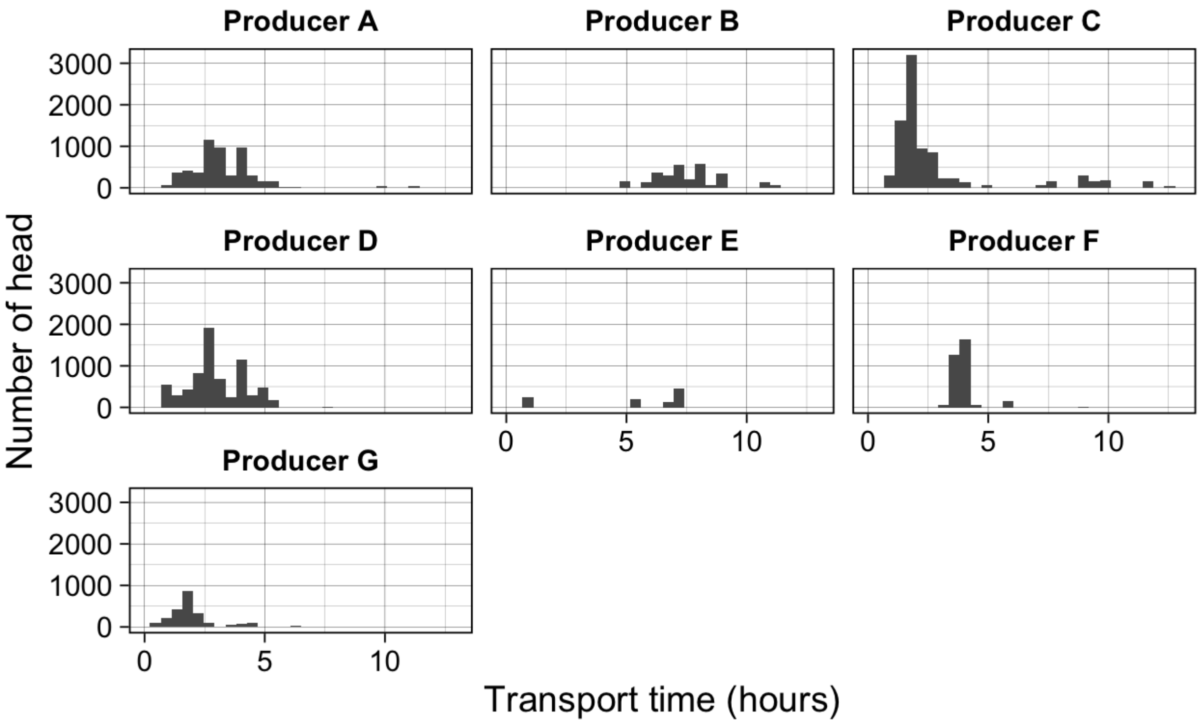

Figure 3: Approximate time in transport (hours) for all producers

Table 13: The effect of transport, lairage and time off feed on the odds of high colour

Predictors	Baseline Model		Lairage Model		Transport Model		Time off Feed Model	
	Odds Ratio	**Significance**	**Odds Ratio**	**Significance**	**Odds Ratio**	**Significance**	**Odds Ratio**	**Significance**
Intercept	0.10	*P* = 0.087	0.03	*P* = 0.015	0.17	*P* = 0.226	0.02	*P* = 0.010
lairage time			1.05	**<0.001**				
transport time					1.00	0.856		
time off feed							1.05	**<0.001**

10.4.2 Climate conditions during lairage

During lairage wind speed, ambient temperature and rainfall influenced the odds of carcasses being classified as high colour (Table 14). There was no relationship between relative humidity or solar radiation and odds of high colour within this model. As ambient temperature increases by 1 degree on day 0, the odds of cattle having high colour decreased by 0.9747 times the odds of cattle with 1 average degree less. As average wind speed increases on day 0 by 1m/s, the odds of arriving cattle being high colour were 1.0278 ($P<0.01$) times the odds of cattle being high in colour when exposed to 1 m/s less. A similar result was shown with wind speed min and max which had an odds ratio of 1.0412 and 1.0139 respectively. Rain on the day of arrival to the processor had a significant effect on the incidence in high colour. Rain on day 0 increased the likely hood of cattle having high colour by 1.23 times the odds of cattle having high colour exposed to 1mm total rain less (Table 14). Temperature humidity index was not significant in when modelled ($P > 0.05$).

Table 14: The odds ratios for the effect of temperature (mean, range, max and min), solar radiation, wind speed, relative humidity and rain during lairage on the incidence of high colour

Predictors	Mean Model		Range Model		Max Model		Min Model	
	Odds Ratio	**Significance**	**Odds Ratio**	**Significance**	**Odds Ratio**	**Significance**	**Odds Ratio**	**Significance**
Intercept	0.0116	$P < 0.001$	0.0094	$P < 0.001$	0.0108	$P < 0.001$	0.0091	$P < 0.001$
SR_{MEAN}	1.0005	0.486	0.9996	0.497			1.0004	0.559
SR_{MAX}					0.9999	0.599		
WS_{MEAN}	1.0278	**0.004**	1.0183	0.074				
WS_{MAX}					1.0139	**0.010**		
WS_{MIN}							1.0412	**0.011**
RH_{MEAN}	1.0050	0.143						
RH_{RANGE}			0.9969	0.340				
RH_{MAX}					1.0002	0.944		
RH_{MIN}							1.0082	**0.003**
$T_{A,MEAN}$	0.9747	**0.011**						
$T_{A, RANGE}$			0.9926	0.431				
$T_{A, MAX}$					0.9800	**0.014**		
$T_{A, MIN}$							0.9918	0.296
Rain	1.2323	**<0.001**	1.2233	**<0.001**	1.2265	**<0.001**	1.2276	**<0.001**

10.5 Processor factors influencing high colour

10.5.1 Time to grading

Time to carcase grading influenced the likelihood of carcasses with high colour. Time to grade was categorised into six categories, encompassing 4 hour periods between 8 and 24 hours, 24 to 48 hours and then ≥ 48 hours (Table 15). As time to carcass grading increased past time category 1 (8 to 12 h) the odds of a carcass being classified as high colour had decreased. Time category 2, 3, 4, 5 and 6 were 0.5401, 0.6573, 0.5310, 0.2991 and 0.2025 times the odds of time category 1 for high colour carcasses based on high colour ($P < 0.001$) (Table 1).

Table 15: Time to carcass grading categories showing total carcasses and proportion carcasses graded within each time category

Time Category	Time to Grading, h	Total Carcasses	Proportion Graded
1	8 h to 12 h	8 179	5.81
2	12 h to 16 h	63 498	45.09
3	16 h to 20 h	19 635	13.94
4	20 h to 24 h	26 969	19.15
5	24 h to 48 h	3 456	2.45
6	≥ 48 h	19 085	13.55

10.6 Carcass factors influencing high meat colour scores

10.6.1 Hot Standard Carcass Weight

Hot standard carcase weight (HSCW) varied between producers, although mean HSCW was 332 ± 60.5 kg (Table 16). Hot standard carcase weight influenced the incidence of high colour, where a 10 kg increase in HSCW the odds of high colour was 0.9046 times the odds of a carcass 10 kg lighter ($P < 0.001$). This is further emphasised when carcasses were grouped by sex and compared (Figure 4). The average HSCW was lower when carcasses had a colour score < 4, irrespective of sex (Figure 4).

Table 16: The mean, median, minimum and maximum HSCW's for cattle from each producer

Item	Producer						
	A	B	C	D	E	F	G
Mean	424 ± 43.2	287 ± 29.5	298 ± 57.2	324 ± 35.3	333 ± 37.5	260 ± 24.4	349 ± 62.6
Median	426	288	281	324	332	259	351
Minimum	196	197	169	102	187	172	61
Maximum	694	381	554	483	471	348	602

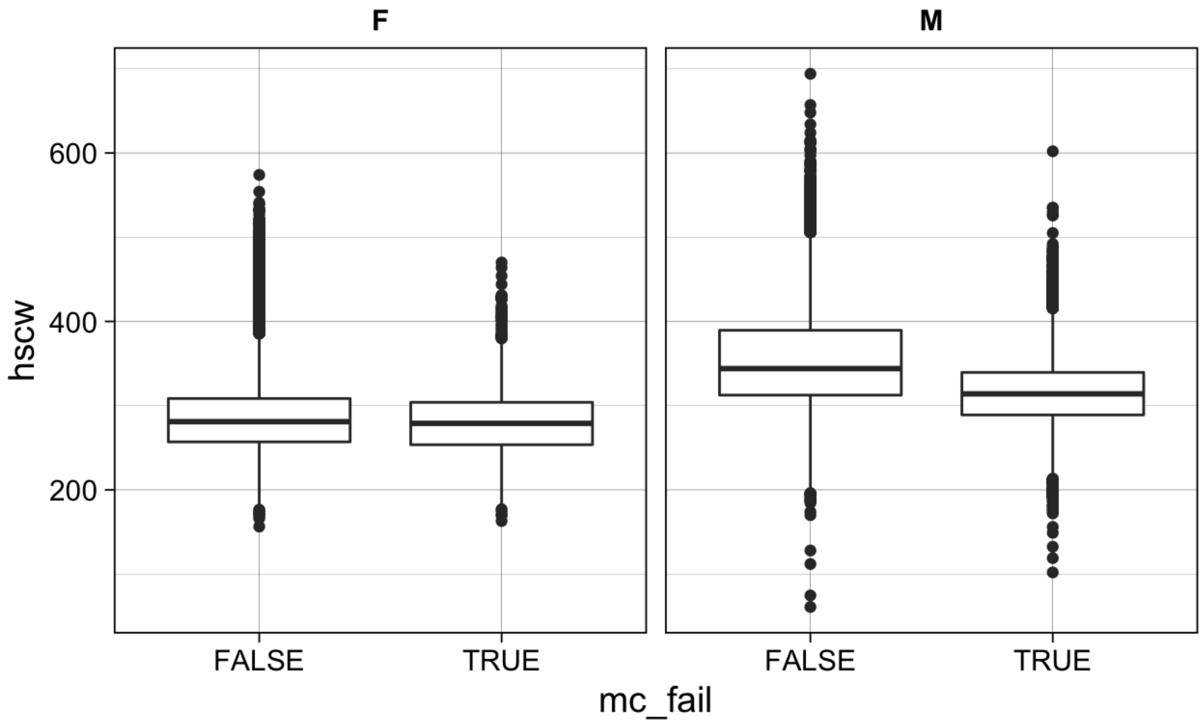

Figure 4: The mean, median, min and max HSCW's for high colour >3 or normal colour <4 for males and females

10.6.2 Ossification

There was some variability in ossification score across the seven producers within this study (Table 17). Ossification was significant in the model (*P* < 0.001; Table 1). As ossification score increased the odds of the carcass being high in colour, where and increase in ossification score by 10 units the odds of having high colour were 1.0634 times the odds compared with a carcass with an ossification score 10 units lower.

Table 17: The mean, median, minimum and maximum ossification scores for cattle from each producer

Item	Producer						
	A	**B**	**C**	**D**	**E**	**F**	**G**
Mean	168 ± 55.5	159 ± 28.6	154 ± 23.4	153 ± 18.4	151 ± 13.2	143 ± 16.9	163 ± 37.4
Median	160	150	150	150	150	140	160
Minimum	100	110	100	100	100	100	100
Maximum	590	400	400	500	250	280	590

10.6.3 Marbling

Producer A had the highest mean marble score with 619 ± 227, however on average marble scores across producers was 387 ± 140 (Table 18).

Marbling was evaluated categorically by group rather than a continuous absolute score in the base model and had a significant effect on the high colour of carcasses. Marbling was significant in the model for the 300 to 500 ($P < 0.001$), 500 to 700 ($P = 0.001$) and > 700 ($P < 0.001$) marbling categories (Table 1). The odds of each marbling group having a high colour, in ascending marble score, were 0.5059 (MSA Marble 300 to 500), 0.6609 (MSA Marble 500 to 700) and 0.4047 (MSA Marble 700+) times the odds of when compared with the lowest MSA marbling category (MSA Marble 100 to 300). Relative to the low marbling group (100-300), the other marbling groups exhibit lower odds of being classified as high colour carcass as marbling score increased.

Table 18: The mean, median, minimum and maximum MSA marble scores for each producer

Item	Producer						
	A	B	C	D	E	F	G
Mean	619 ± 227	380 ± 56	392 ± 72	351 ± 54	353 ± 48	358 ± 52	301 ± 113
Median	560	360	360	350	350	350	300
Minimum	100	210	120	100	100	180	100
Maximum	1 190	590	980	1000	780	600	1050

10.6.4 Rib fat

The average rib fat across the seven producers was 15.6 ± 3.04 mm. Cattle from Producer A general had higher rib fat coverage in comparison to the other producers (Table 19). Rib fat was significant in the model ($P < 0.001$) and had a positive influence on normal colour (Table 1). As rib fat increased by 1 mm the odds of a carcass being classified as high colour was 0.8636 times the odds of a carcass with a 1 mm reduction in rib fat depth. Higher means were seen in the normal colour category's for both male and female carcasses (Figure 5). Any score of 30 mm rib fat or more was only captured under the normal colour category for females and males had no scores over 25 mm with a high colour.

Table 19: The mean, median, minimum and maximum rib fat scores (mm) for each producer

Item	Producer						
	A	B	C	D	E	F	G
Mean	11.5 ± 4.5	7.43 ± 2.0	7.59 ± 2.5	9.08 ± 3.4	9.92 ± 3.7	6.22 ± 1.5	8.74 ± 3.6
Median	10.0	7.0	7.0	8.0	9.0	6.0	9.0
Minimum	3.0	1.0	2.0	1.0	1.0	2.0	1.0
Maximum	55.0	25.0	56.0	60.0	32.0	40.0	55.0

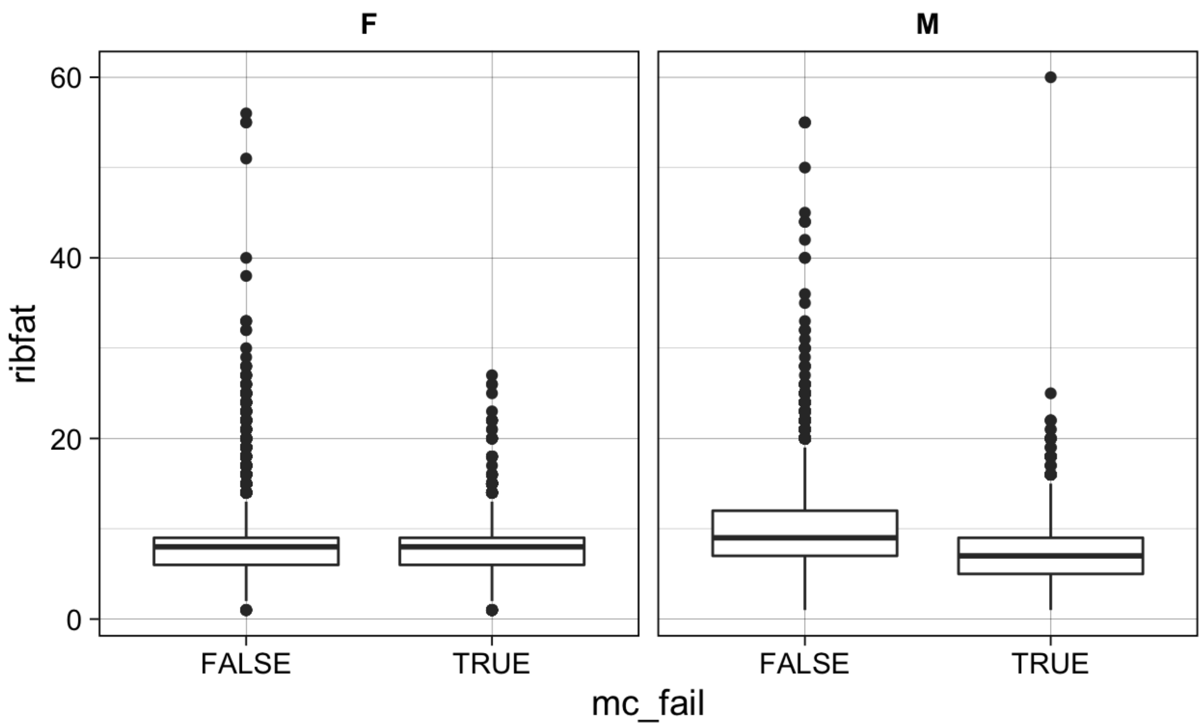

Figure 5: The mean, median, min and max rib fat mm for high colour (True) or normal colour (False) for males and females

10.6.5 Hump Height

The average hump height from producers was 61.3 ± 17.7 mm, although Producer G had the highest average hump height (Table 20). Hump height was significant in the model (*P* < 0.001; Table 1). As hump height increased by 10 mm the odds of a carcass being classified as high colour was 0.9458 times the odds of a carcass with a hump measuring 10 mm less.

Table 20: The mean, median, minimum and maximum hump heights (mm) for each producer

Item	Producer						
	A	B	C	D	E	F	G
Mean	66 ± 18.1	49 ± 9.2	51 ± 12.2	64 ± 17.3	66 ± 14.9	47 ± 9.1	67 ± 19.9
Median	65.0	45.0	45.0	60.0	65.0	45.0	65.0
Minimum	15.0	20.0	20.0	15.0	30.0	20.0	20.0
Maximum	160	150	140	265	220	170	280

10.6.6 Fat Colour

Relative to fat colour 0, animals with higher fat colours were more likely to have high colour. Fat colour was significant in the model (*P* < 0.001; Table 1) with the odds of fat colour groups 1, 2, 3 and 4+ being2.5632, 2.3686, 3.7399 and 5.0897 times more likely to be classified as high colour than the odds of fat colour group 0. This was reflected in the proportion of high colour carcasses for each fat colour group (Table 21).

Table 21: Total number of carcasses and percentage high colour for each fat colour group

Colour	Total Carcasses	Normal Colour	High Colour	Proportion Non-Compliant
0	37 244	35485	1759	4.7%
1	67 644	62104	5540	8.2%
2	33 261	29651	3610	10.9%
3	2 296	1892	404	17.6%
4 +	377	298	79	21.0%

11 Discussion

11.1 Animal factors influencing dark cutting

11.1.1 Hormone Growth Promotants

The use of HGP within this study were associated with an increased risk of both pH carcass failures and high colour. The effect was smaller on high colour compared to high pH with odds ratios of 1.483 and 2.2927 respectively.

11.1.2 Sex

Females had a higher incidence of high colour, when compared with steers in the raw data, and this was reflected in the model for high colour with an odds ratio of 0.9638. This is similar to many other studies that also found steers to have lower incidences of dark cutting than females (Voisinet *et al.* 1997a; Wulf *et al.* 1997; Scanga *et al.* 1998; Warren *et al.* 2010; Romero *et al.* 2013). This was different to the results for high pH which appeared to be confounded in the pH models as it varied from the raw data with a slight increase in high pH odds ratio of 1.1458.

11.1.3 Days on feed

Increasing days on feed increased was associated with a slight increase in both the incidence of pH non-compliance and high colour in this study, with odds ratios of 1.0199 and 1.0396 respectively.

11.1.4 Feedlot pulls

Feedlot pulls were associated with a similar increased incidence of pH non-compliance and high colour in the current study.

11.2 Weather factors influencing dark cutting

Climatic variables during the 7 days prior to feedlot exit were identified as risk factors for pH non-compliance and high colour within this study. These results were very similar as expected with the high correlation between pH and colour in the study. However there were some differences, relative humidity was a significant effect for high colour only. This was only a small effect with an odds ratio of 1.0119 for an increase in high colour with increasing relative humidity. Solar radiation average was significant for high pH and not high colour, while solar radiation max was significant for only high colour.

11.3 Lairage factors influencing dark cutting

11.3.1 Transport time

Transport time did not increase the likelihood of pH non-compliance or high colour in the study (P>0.5).

11.3.2 Time in Lairage

Increasing the time cattle spent in lairage was associated with an increase in high colour and pH non-compliance. The results for both the high colour and high pH models were almost identical with odd ratios of 1.05 and 1.06 respectively.

11.3.3 Climatic conditions during lairage

During lairage there was no relationship between relative humidity, ambient temperature or solar radiation and odds of pH non-compliance. However relative humidity min and ambient temperature (mean, max and min) were significant for high colour. This indicates that temperature may have a greater effect on muscle colour than pH at grading. Increasing wind speed and rainfall increased odds of pH non-compliance and high colour.

11.4 Processor factors influencing dark cutting

11.4.1 Time to grading

Time between slaughter and grading had differing effect sizes on both high pH and high colour at grading. The reduced odds of having high colour with increased time to grading was greater than the reduction in odds of having high pH, with 12-16h (0.5401, 0.6910), 16-20h (0.6573, 0.9497), 20-24h (0.5310, 0.6984), 24-48h (0.2991, 0.4147) and 48+h (0.2025, 0.6450) respectively.

11.5 Carcass factors influencing dark cutting

11.5.1 Hot standard carcass weight, marbling and rib fat

Findings from this study show that heavier carcasses, with higher MSA marbling scores and rib fat depths have lower incidences of pH non-compliance and high colour.

11.5.2 Fat colour

The fat colour effect on the incidence of pH non-compliance and high colour were similar in this study. Fat colour groups 2-4+ were very similar between the high pH and high colour models. However fat colour group 1 had markedly higher odds ratio for high colour than high pH, 2.5632 compared with 1.9728.

11.5.3 Ossification

Ossification had negative effect on both high pH and colour in both models with very similar odds ratios 1.0548 and 1.0634 respectively.

11.5.4 Hump height

As hump height increased both high pH and high colour decreased with similar odds ratios in both models, with odds ratios 0.9458 and 0.9504 respectively.