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Experiment 3. Time to grading and re-grading analysis and economic analysis

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Abstract

The aims of this study were to determine (i) whether the time from slaughter to grading influences meat colour and pH of grain-fed carcasses, (ii) to determine which carcass characteristics influence meat colour and pH, (iii) to determine the viability of regrading at later time intervals as a method to improve meat colour and pH to minimise the incidence of dark colour and high pH in grain-fed carcasses in Australia and (iv) to conduct an economic analysis for the regrading. Time of grading analysis conducted to 139,703 grain-fed carcasses from seven feedlots and processed in three plants between September 2017 and August 2018 demonstrated similar incidences of Dark Colour-High pH meat among plants (2.8 %) and that earlier time of grading (< 24 h) was associated with higher percentages of Dark Colour-Normal pH meat. Then, grading analysis of 1,181 carcasses demonstrated that the incidence of Dark Colour-Normal pH and Dark Colour-High pH meat had an inverse relationship with Temp@pH6, and that reduced P8Fat, marbling score, HSCW, and time of grading all were associated with high percentages of Dark Colour-Normal pH meat. Finally, when regrading Dark-Colour-High pH carcasses, 81.8 % of those carcasses showed a reduction in pH and 7.07 % in colour. The incidence of high pH non-compliance drops from 8.4% at grading (~12 hrs post-slaughter) to 5.7% at regrading (~23 hrs post-slaughter). Our results suggest that when carcasses are graded at about 12 hrs post-slaughter, meat colour and pH may have not reached their ultimate value and that regrading or delaying the grading helps to reduce muscle ultimate pH and to improve meat colour reducing the incidences of high pH. When grading occurs early, the net economic benefit of regrading 12 hrs later, including labour costs, was calculated to be $5,129 over 1,000 beef carcasses.

Executive summary

High pH in beef carcasses is a complex multifactorial condition influenced by pre- and post-slaughter factors including abattoir management and processing. In Australia, a carcass is classified as high pH when the meat pH at grading of the m. longissimus thoracis (**LT**) measured 8- 48 hours post-slaughter at the quartering site is greater than 5.70. This condition is normally associated with the AUS-MEAT colour score > 3. However, it has been observed that dark-colour meat in grain-fed cattle is not always accompanied by high pH at grading. Furthermore, previous studies have demonstrated that the LT of beef carcasses do not reach the ultimate colour and pH until at least 24 h post-slaughter. The Therefore, the objectives of this study were to determine whether the time from slaughter to grading influences meat colour and pH of grain-fed carcasses, to determine which carcass characteristics influence meat colour and pH at grading, and to determine the viability of regrading at later time intervals as a method to improve meat colour and pHu to minimise the incidence of dark colour and high pH carcasses in Australia.

Between September 2017 and August 2018, the time of grading of 139,703 grain-fed carcasses from seven feedlots and processed in three plants were obtained from the MSA feedback data set provided by MLA. Time of grading was defined as **< 24 h** if carcasses were graded the same day of slaughter; **1 day** if carcasses were graded the following day (within 24 h post-slaughter); **2 days** if carcasses were graded within 48 h post-slaughter; **3 days** if carcasses were graded within 72 h post-slaughter; **4 days** if carcasses were graded within 96 h post-slaughter; and **5 days** if carcasses were graded within 120 h post-slaughter.

From the MSA feedback data, carcasses where classified into three categories **Dark Colour-High pH** if a carcass had AUS-MEAT colour score > 3 and pH > 5.70 at grading; **Dark Colour-Normal pH** if a carcass had AUS-MEAT colour score > 3 and pH ≤ 5.70 at grading; and **Normal Colour-High pH** if a carcass had AUS-MEAT colour score ≤ 3 and pH > 5.70 at grading. Data were analysed to obtain the proportion of carcasses graded at different time, to obtain the average pH and loin temperature at grading, and to obtain the proportion of carcasses from each category according to the time to grading.

Between September 2017 and August 2018, Plant A and B were visited once per month for 3-5 days, and fortnightly during the 12-week period from January to March 2018. Plant C was visited twice during the experimental period. Fifty (50) carcasses per day were assessed for determination of pH-temperature decline, and grading and regrading. A total of 2,454 carcasses were assessed for grading and pH-temperature decline; 1,357 carcasses from plant A, 897 carcasses from plant B, and 200 carcasses from plant C. Of those 1,357 carcasses from plant A, 1,181 carcasses were assessed for re-grading.

Grading was conducted as per MSA protocols and according to each processing plant timing by accredited MSA graders after cutting at the quartering site and after at least 30 minutes of blooming. In addition, objective colour determination was conducted using a NIX sensor (Nix Pro Colour Sensor™, Nix Sensor Ltd, Burlington, Ontario, Canada) immediately after visual colour assessment at grading. Objective colour determination was obtained from 1,828 carcasses; 1,041 carcasses from plant A, 587 carcasses from plant B, and 200 carcasses from plant C.

Regrading was only possible in Plant A. Regrading was conducted as per grading protocols with the help of an accredited grader on the opposite side of the carcass after at least 30 minutes of blooming. In addition, objective colour determination was conducted using a NIX as per grading immediately after visual colour assessment.

Data was collected from the MSA feedback and from each visit to statistically determine whether the time from slaughter to grading influences meat colour and pH of grain-fed carcasses, and the viability of re-grading at later time intervals as a method to improve meat colour and pH. Data were analysed to determine differences in pH, objective colour values and AUS-MEAT colour score between grading and regrading and between plants. Prediction models were used to estimate the carcass factors influencing the proportion of carcasses presenting Dark Colour-Normal pH and Dark Colour-High pH. Temp@pH6 and incidences of heat and cold shortening were analysed for each plant and were associated to the incidences of Dark Colour-Normal pH and Dark Colour-High pH meat. The relationship between L*, a*, b*, Hue angle and Chroma, and AUS-MEAT colour score for grading and regrading was determined to then obtain a prediction of AUS-MEAT colour score from objective colour values. Finally, differences between Dark Colour-Normal and Dark Colour-High pH in objective colour values were determined.

Between 84 and 89 % of carcasses were graded within 24 h post-slaughter in all processing plants. Plant A, B and C had a minimum grading time of < 24 h post-slaughter for 85.1 % of carcasses, 24 h post-slaughter for 83.8 % of carcasses, and 24 h post-slaughter for 84.8 % of carcasses, respectively. There was a high percentage of **Dark Colour-Normal pH** carcasses (10.6 %) in cattle slaughtered at plant A while the incidence of this category was low in plant B (0.24 %) and zero in plant C. By contrast, the proportion of carcasses showing **Dark Colour-High pH** was similar among plants (2.8 % on average). There were no significant differences in the average pH at grading between plant A and B (5.56) but it was lower for plant C (5.52 SED ± 0.004; P < 0.001). There were significant differences in the average loin temperature at grading between plants, being higher for plant A, followed by plant B and C (10.0, 6.5, and 6.1 ± 0.05 ˚C).

There was a high percentage of **Dark Colour-Normal pH** (11.6 %) when carcasses were graded < 24 h post-slaughter. By contrast the incidence of carcasses showing **Dark Colour-High pH** was similar among grading times < 24 h, 1, and 2 days post-slaughter, while grading carcasses more than 3 days post-slaughter showed the lowest incidence of this category. There were significant differences in the average pH at grading among grading times, it was higher < 24 h and 2 days post-slaughter (5.56) and it was lower at 120 h post-slaughter (5.50 ± 0.004; P <0.001).

For plant A, carcasses graded within the first 24 h post-slaughter had higher incidence of **Dark Colour-Normal pH** (25.5 %) compared to carcasses graded more than 1-day post-slaughter. The incidence of carcasses showing **Dark Colour-High pH** was higher 4 days post-slaughter (6.28 %) while the lowest incidence was observed < 24 h post slaughter (2.6 %). For plant B, by contrast, carcasses graded 24 h post-slaughter had higher incidence of **Dark Colour-High pH** (3.62 %) compared to carcasses graded more than 1-day post-slaughter. The incidence of carcasses showing **Dark Colour-Normal pH** was very low at all grading times. For plant C, carcasses graded 24 h post-slaughter had higher incidence of **Dark Colour-High pH** (2.79 %) compared to carcasses graded < 24 h and 2 days post-slaughter, while the incidence in carcasses graded more than 3 days post-slaughter was minimal.

Plant A had high incidences of cold shortening (4.2 %) compared to other plants, but incidences of heat shortening were similar between plant A and B (2.6 and 3.0%). For plant A, it was demonstrated that the incidences of **Dark Colour-High pH**, and **Dark Colour-Normal pH** carcasses were inversely related to the Temp@pH6 (P <0.001) and associated to cold shortening. Temp@pH6 < 12 ˚C was predicted to have high incidences of **Dark Colour-High pH** carcasses.

Prediction model indicated that reduced rump fat thickness, reduced marbling scores and lighter carcasses all were associated with higher percentages of **Dark Colour-Normal pH** meat in plant A (P < 0.05) and that longer time from slaughter to grading was associated with lower percentages of dark colour meat and lower percentages of **Dark Colour-Normal pH** carcasses.

Grading at plant A was normally conducted between 19:30 and 2:30 AM, while at plant B it was conducted between 6:00 AM and 10:00 AM, and in plant C between 2:00 AM and 6:00 AM. Grading at plant A was conducted by three graders and regrading was conducted between 4:00 and 5:00 AM with the help of a 4th different grader. Grading at plant B and C was conducted by five and three different graders, respectively. In general, there were no differences between graders in each plant for objective colour values. However, in plant A, differences were observed between graders in L* value (grader 1= 36.65, grader 2 =36.13, grader 3=34.63, and grader 4= 36.09 ± 0.8623; P = 0.003). At grading and regrading, there were differences in all objective colour values between AUS-MEAT colour scores (P <0.001 for all), and there was an inverse relationship between the degree of darkness and the objective colour values.

Objective colour values were different between grading and regrading, being higher at regrading (P < 0.001). However, differences determined by objective colour determination were not associated with differences reported by the grader. At regrading, the grader consistently reported darker meat colour (AUS-MEAT colour score > 3). There were significant differences in objective colour values between carcass categories such that **Dark Colour-Normal pH** carcasses had higher values for L*, a*, b*, and Chroma than **Dark Colour- High pH carcasses.**

At grading, the incidence of dark colour carcasses (AUS-MEAT colour score >3) was 30 %, and the average pH of carcasses showing AUS-MEAT colour score 4 was 5.60. This indicates that there was a high proportion of carcasses showing AUS-MEAT colour score 4 but normal pH at grading (91 %). At regarding, the average pH of carcasses showing AUS-MEAT colour score 4 was 5.51 indicating a reduction in pH from grading.

For pH, 77.7 % of all graded carcasses showed a reduction of pH when regraded at 21.1 h post-slaughter independently of their pH at grading. The mean pH at grading was 5.59 ± 0.195 (min 5.36, max 6.79) and the mean pH at regrading was 5.52 ± 0.186 (min 5.29, max 6.81; t = 7.95 d.f= 2080 , P < 0.001). For meat colour, 45.6 % of all graded carcasses showed an increment in AUS-MEAT colour score when regraded at 21.1 h post-slaughter independently of their original colour score at grading. The mean colour score at grading was 3.04 ± 1.32 (min 1 max 6) and the meat colour score at regrading was 3.4 ± 0.918 (min 1, max 7; t =-7.19 d.f=1845.65. P < 0.001).

When regrading non-compliant carcasses (**Dark Colour-High pH**), 81.8 % of those carcasses showed a reduction in pHu and 32.3 % and became complaint in pH, and 7.07 % became compliant in colour (AUS-MEAT colour score ≤ 3). When regrading **Dark Colour-Normal pH** carcasses, 57.8 % of them showed a reduction in AUS-MEAT colour score at regrading and 34.8 % showed colour score ≤ 3. Nevertheless, when compliant (normal pH and normal colour) carcasses were regraded, 17.5 % and 61.6 % of them showed an increment in pH and AUS-MEAT colour score, respectively. Indeed, 29.3 % had meat colour darker than AUS-MEAT 3.

The incidence of high pH non-compliance drops from 8.4% at grading (~12 hrs post-slaughter) to 5.7% at regrading (~23 hrs post-slaughter). Our results suggest that when carcasses are graded at about 12 hrs post-slaughter, meat colour and pH may have not reached their ultimate value and that regrading or delaying the grading helps to reduce muscle ultimate pH and to improve meat colour reducing the incidences of high pH. When grading occurs early, the net economic benefit of regrading 12 hrs later, including labour costs, was calculated to be $5,129 over 1,000 beef carcasses.

These results demonstrated that earlier time of grading and reduced P8Fat, marbling score, and HSCW all were associated with higher percentages of **Dark Colour-Normal pH** and that the incidence of **Dark Colour-Normal pH** and **Dark Colour-High pH** meat had an inverse relationship with Temp@pH6. Our results suggest that when carcasses are graded during the first 24 h post-slaughter, meat colour and pH may have not reached their ultimate value and that regrading or delaying the grading helps to reduce muscle ultimate pH and to improve meat colour reducing the incidences of high pH.

These results also suggest that, although graders are MSA certified, they potentially differ in colour perception. We suggest, therefore that the industry must use objective colour measurement to confirm the real variation in colour between grading and regrading based on colourimetry data (eg. Chromameters such as NIX, Minolta or Hunterlab). We also suggest further studies to determine the metabolic and meat quality differences between **Dark Colour-Normal pH** and **Dark Colour-High pH** meat because, despite being MSA graded, their quality could be inferior to normal colour and normal pH carcasses.

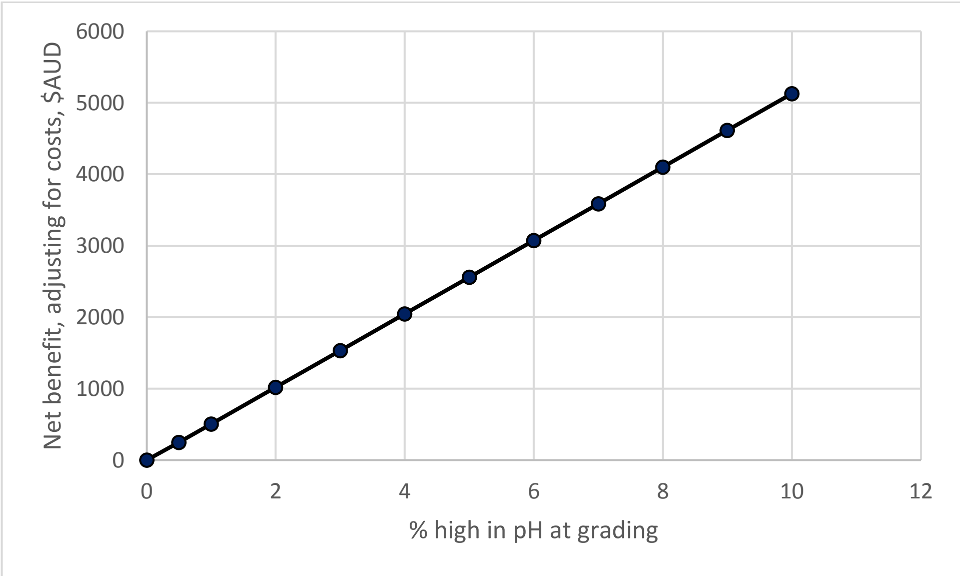
Recommendations

- Where possible, standardize grading time to more than 20 h post-slaughter to reduce the incidences of Dark Colour-Normal pH and Dark Colour- High pH meat.
- Where grading occurs <20 h post-slaughter, any carcasses graded as high pH must be re-graded.
- Delay the time of grading or regrading for those carcasses showing reduced P8Fat, marbling score, or HSCW, because they may be associated with Dark Colour-Normal pH meat, or ensure 'truly effective' electrical stimulation is in place.
- Avoid low Temp@pH6 or cold shortening because this may be associated with Dark Colour-Normal pH and Dark Colour- High pH meat, ie. Ensure carcasses are in the MSA pH-temperature window.
- The industry should consider using objective colour measurement to confirm the real variation in colour between grading and re-grading based on colourimeter data, using objective measurements and a chromameter (NIX, Minolta or Hunter lab).
- Further consideration should be given to handling of meat colour chip standards and training of AUS-MEAT/MSA graders, to ensure standardisation between graders.
- The improvement in colour in many beef loins over the period 8-48 h post-slaughter needs to be recognised and the mechanisms investigated, to allow increased compliance to company specifications for colour.
- Further studies are required to determine the metabolic and meat quality differences between Dark Colour-Normal pH and Dark Colour-High pH meat because, despite being MSA graded, their quality could be inferior to normal colour and normal pH carcasses.
- Further studies are required to determine the effects of spray chilling on meat pH and colour and on the incidences of Dark Colour-Normal pH and Dark Colour-High pH meat.
- The economic cost of high pH beef carcasses, and the benefit of regrading, should be communicated to the meat industry, via an industry fact sheet.

Key messages

- Processors should standardize grading time to more than 20 h post-slaughter to reduce the incidences of Dark Colour-Normal pH and Dark Colour- High pH meat.
- Where delaying grading to >20 h post-slaughter is not possible, any carcasses with high pH should be regraded at >20 h.
- Processors should delay the time of grading or should implement regrading of those carcasses showing reduced P8 Fat, marbling score, and HSCW, because they may be associated with of Dark Colour-Normal pH meat.
- Processors should control the chilling process to avoid low Temp@pH6 or cold shortening because they may be associated with Dark Colour-Normal pH and Dark Colour- High pH meat.
- Beef loins that are non-compliant for colour, but compliant for pH (Dark colour-Normal pH) at early times (<18 h) of grading are very likely to become compliant for colour if graded at 48 h post-slaughter.

- If adopting these measures to regrade beef carcasses, and the incidence of high pH at early grading (12 h post-slaughter) is 10%, the net economic benefits of regrading are $5,129, over 1,000 carcasses.



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1 Background

High pH in beef carcasses is a complex multifactorial condition influenced by pre- and post-slaughter factors including animal nutrition and temperament, duration of transport, seasonality, and abattoir management and processing (Knee *et al.* 2004; McGilchrist *et al.* 2014). In Australia, a carcass is classified as high pH when the meat pH at grading of the m. longissimus thoracis (**LT**) measured 8 - 48 hours post-slaughter at the quartering site, at grading, is greater than 5.70. This condition is normally associated with AUS-MEAT colour score > 3 (AUS-MEAT, 2005).

Meat colour is an important determinant of consumer acceptance of meat and meat products. However, the perception of meat colour is affected by animal and processing factors. Meat colour assessment can be done using visual or instrumental approaches. In Australia, meat colour of beef carcasses is assessed visually 8- 48 hours post-slaughter using AUS-MEAT standard meat colour chips where 1a = palest and 7 = darkest. If the carcasses have been 'effectively' electrically stimulated, the carcasses can be graded at 8 or more hours post-slaughter (Australian-meat-industry-classification-system, 2018). In the absence of 'effective' electrical stimulation, the grading must occur after 18 hours post mortem (Australian-meat-industry-classification-system, 2018). The caveat here is that there is a lack of definition of 'effective' electrical stimulation, although a good starting point would be to ensure the carcasses are in the correct part of the pH-temperature window, hence not cold- or heat-shortened (temperature at pH= 6 of <12oC and <35oC respectively; Gutzke et al., 2014). Meat is graded as dark when meat colour score is > 3 (AUS-MEAT, 2005). Even though this assessment is carried out by AUS-MEAT trained and certified graders, the colour perception is still subjective.

Although from 2017 meat colour is no longer a minimum Meat Standard Australia (MSA) requirements, the industry specification in order to carcasses to be eligible for the cyphers GF (grain-fed) and GFYG (grain-fed young beef) the AUS-MEAT colour score must be 1 a-b-c to 3 (AUS-MEAT, 2019) hence it still represents a significant economic loss for producers and processors. It has been observed that dark-colour meat in grain-fed cattle is not always accompanied by high pHu and that dark meat associated with normal pH is tougher than lighter meat (Mahmood *et al.* 2017). Previous studies have demonstrated that the LT muscles of beef carcasses chilled by traditional methods, do not reach ultimate colour and pH until at least 24 h post slaughter (Fabiansson *et al.* 1984; Murray 1989) and that differences in post-mortem muscle metabolic activity (eg. glycogen utilization rate) and mitochondrial metabolism are causative factors for dark colour meat in grain-fed beef carcasses (Mahmood *et al.* 2017).

Therefore, the objectives of this study were to determine whether the time from slaughter to grading influences meat colour and pH of grain-fed carcasses, to determine which carcass factors influence meat colour and pH at grading, and to determine the viability of regrading at later time intervals as a method to improve meat colour and pHu to minimise the incidence of dark colour and high pH carcasses in Australia.

2 Project objectives

1. Conduct a 12-month slaughter chain audit of grain-fed cattle at a minimum of two processors with a known high incidence of high pH, and their supplying feedlots, to determine factors contributing to variation in high pH.
2. Determine whether time to grading influences meat colour of grain-fed carcasses, and the viability of re-grading at later time intervals as a method to improve meat colour and pH.
3. Make recommendations to minimise the incidence of high pH carcasses in Australia

3 Methodology

3.1 Time from slaughter to grading analysis

Between September 2017 and August 2018, the time of grading of 139,703 grain-fed carcasses corresponding to three (3) processing plants (*A, B,* and *C*) was obtained from the MSA feedback data set provided by MLA. Seven (7) feedlots (*G, H, I, J, K, L, M*) delivering cattle to those processing plants were selected to participate in this study. Table 3.1 shows the processing plant destination of cattle from each feedlot. Animals recorded as grass-fed in the original MSA feedback data set were removed from this analysis.

Table 3.1. The delivery of cattle from each feedlot to each processing plant during the study

	Feedlot G	Feedlot H	Feedlot I	Feedlot J	Feedlot K	Feedlot L	Feedlot M
Plant A			x	x	x		
Plant B		x	x			x	
Plant C	x		x	x			x

Because the exact time of slaughter and time of grading (in hours and minutes) is not normally recorded, and is not available from the MSA feedback; time to grading was defined as **< 24 h** if carcasses were graded the same day of slaughter; **1 day** if carcasses were graded the following day (within 24 h post-slaughter); **2 days** if carcasses were graded within 48 h post-slaughter; **3 days** if carcasses were graded within 72 h post-slaughter; **4 days** if carcasses were graded within 96 h post-slaughter; and **5 days** if carcasses were graded within 120 h post-slaughter.

From the MSA feedback data, carcasses where classified into three categories **Dark Colour-High pH** if a carcass had AUS-MEAT colour score > 3 and pH > 5.70 at grading; **Dark Colour-Normal pH** if a carcass had AUS-MEAT colour score > 3 and pH ≤ 5.70 at grading; and **Normal Colour-High pH** if a carcass had AUS-MEAT colour score ≤ 3 and pH > 5.70 at grading.

The electrical input characteristics for each processing plant are shown in Table 3.2. The average time of stimulation for each operational unit was measured by the project personnel. These are discussed in the results.

Table 3.2. Electrical inputs at each stage of the processing floor for each processing plant. Note that data was not available for the hide puller specifications a Plant C.

PLANT	Halal	Time from stunning to scan NIRS (~mins)	Electrical Input	Pulse width (ms)	Period (ms)	Frequency (Hz)	Current (mA)	Average time (s) of stimulation
A	NO	30	Immobiliser	NP[1]	NP	NP	NP	NP
			Estim- Bleed[3]	500	333	3	3	0:22:59
			Hide Puller	NA[2]	25	40	Peak 2.5 A (average 300 mA after 1 ms and zero after 2ms)	0:05:22
B	YES	40	Immobiliser	100	500	2000	1000	0:09:26
			Estim- Bleed	500	200	5	400	0:15:03
			Hide Puller	NA	24		4 A to 500 mA (after 1 ms) and zero after 2ms)	0:15:03
C	NO	30	Immobiliser	8 (ms) 100 v (PEAK)	68	14.8	1.2 A (leading edge) to 1.1 A (trailing edge)	0:12:00
			Estim- Bleed	500	200	5	600	0:32:51
			Hide Puller	NA	NA	50	NA	0:07:45

[1] NP = Not present, [2] NA = Not available, [3] Estim-Bleed is electrical stimulation applied for purposes of blood removal

3.1.1 Statistical analysis

For statistical analysis, categorical colour score data was converted into factors. Due to the low frequency of some colour categories, AUS-MEAT colour scores 1A, 1B, and 1C were converted to a value = 1. Data were analysed using descriptive statistics and Restricted Maximum Likelihood (REML) using GENSTAT 18 to obtain the proportion of carcasses graded at different time, to obtain the average pH and loin temperature at grading by plant and feedlot, and to obtain the proportion of Dark Colour-High pH, Dark Colour-Normal pH, and Normal Colour-High pH carcasses according to the time to grading for each feedlot and each processing plant. Fixed terms were grading time, plant, and feedlot and random terms were body number and kill day.

3.2 Grading and regrading analysis

Between September 2017 and August 2018, Plant A and B were visited once per month for 3-5 days (dependent on when feedlot cattle were procured each week), and fortnightly during the 12-week period from January to March 2018. Plant C was visited twice during the experimental period due to its low high pH incidences.

Fifty (50) carcasses per day were assessed for determination of pH-temperature decline, and grading and regrading. the pH-temperature decline was conducted by inserting the pH and temperature probes in the m. longissimus lumborum (**LL**) in proximity to the 2nd and 5th lumbar vertebrae on each individual carcass after carcasses enter to the chiller- approximately 1 hour after slaughter. Then, 3 to 4 hours post-slaughter a second measurement of pH and temperature were obtained when the muscle pH was below pH 6. For each measurement and to ensure the accuracy of data collection, the pH and temperature probe was inserted in a fresh incision of the LL. A TPS WP-80M pH meter (TPS Pty Ltd, Brendale, Queensland, Australia) with a polypropylene spear-type gel electrode (Iodone IJ44, TPS Pty Ltd) and a temperature probe that allows temperature compensation, was used to measure pH and temperature. The pH meter and electrode were calibrated at ambient temperature using buffers pH 4.00 and pH 7.00 and recalibrated every 2 hours during the determination of pH-temperature decline. The temperature probe was calibrated twice a month during the sampling period.

Grading was conducted as per MSA protocols and according to each processing plant timing by accredited MSA graders after cutting at the quartering site and after at least 30 minutes of blooming. Grading at plant A was normally conducted between 19:30 and 2:30 AM (12.6 ± 1.15 h after slaughter), while at plant B it was conducted between 6:00 AM and 10:00 AM (21.9 ± 2.57 h after slaughter), and in plant C between 2:00 AM and 6:00 AM (17.2 ± 2.57 h after slaughter). Table 3.2 shows the electrical inputs used at each plant, where the data was available. Each plant had electrical stimulation for bleeding installed but the current and time of application had large variation for this, and other electrical inputs. Project investigators (Dr Paula Gonzalez-Rivas and Mr Cameron Steel), when allowed, followed the graders during grading and recorded pH and temperature at grading, otherwise, pH, loin temperature and meat colour were obtained from the MSA feedback.

In addition, to obtain objective characterization of the subjective colour scores, objective colour determination was conducted using a NIX sensor (Nix Pro Colour Sensor™, Nix Sensor Ltd, Burlington, Ontario, Canada) with 15.0 mm aperture, 45/0° measuring geometry, illuminant D65, and 10° as standard observer settings. Seven measurements were done across the exposed rib eye as described in Holman *et al.* (2018) immediately after visual colour assessment at grading. This device allows the measurement of CIELab (lightness [L*], redness [a*], and yellowness [b*]), and the calculated LCH (ab) values Croma (C*) and Hue Angle (H˚) for each carcass (CIE, 1978).

Regrading was only possible in Plant A due to its early time of grading (~12 h post-slaughter) and boning (~5 AM) that allowed an extra pH and temperature determination after grading and before carcasses were moved to the boning room. Carcasses in Plant B and C were normally graded immediately before being sent to the boning room, therefore there were no chances for regrading in those plants due to lack of chiller space and time.

Carcasses from animals slaughtered on Friday and graded after the weekend were included in the data collection for grading and pH-temperature decline, but those carcasses were not regraded. Project investigators (Dr Paula Gonzalez-Rivas and Mr Cameron Steel) conducted regrading. Regrading was conducted as per grading protocols with the help of an accredited grader on the opposite carcass side and at after least 30 minutes of blooming. In addition, objective colour determination was conducted using a NIX as per grading immediately after the visual colour assessment.

3.2.1 Statistical analysis

Temp@pH6 was calculated using a 2-point interpolation method described by Hwang and Thompson (2001).

$$Temp@pH6=TempA-[\ [(pHA-6) \times (TempA-TempB)\ ]\ \div (pHA-pHB)\ ]$$

TempA and pHA represent the first temperature and pH measurement above pH 6 and TempB and pHB represent the first measurement taken below pH 6. A carcass was defined as being in high rigor temperature or *Heat Shortening* if the Temp@pH6 was > 35˚C or *Cold Shortening* if Temp@pH6 was < 12˚C. Data was collected from the MSA feedback and from each visit to statistically determine whether the time from slaughter to grading influences meat colour and PH of grain-fed carcasses, and the viability of re-grading at later time intervals as a method to improve meat colour and pH.

For statistical analysis, categorical colour score data was converted into factors. Due to the low frequency of some colour categories, AUS-MEAT colour scores 1A, 1B, and 1C were converted to a value = 1. Data were analysed using GENSTAT 18, using descriptive statistics, a t-test to determine differences in pH, objective colour values and AUS-MEAT colour score between grading and regrading, and REML to determine differences in pH, loin temperatures, and objective colour scores between plants and graders. Fixed terms were AUS-MEAT colour score, plant and grader, and the random model was body number and date of killing.

A Generalized Lineal Mix Model (GLMM) was used to estimate the factors influencing the proportion of carcasses presenting Dark Colour-Normal pH and Dark Colour-High pH. This approach used a logit-transformation and binomial distribution of the data witch, by using additive models, logits were predicted as a linear function of fixed and random effects. Fixed effects were, time from slaughter to grading, time from slaughter to regrading and time from grading to regrading, Ema, Epbi, Hgp, Hump

Cold, MSA marbling, Days on Feed, P8Fat (or Rib fat), Sex, Total HSCW, and Temp@pH6. Random factors were Day of Kill, Chiller, Lot, and Grader. Only significant factors were included in the final model.

In order to determine the relationship between L*, a*, b*, Hue angle and Chroma, and AUS-MEAT colour score and to determine the difference between grading and regrading in colour score, a REML analysis was conducted, fixed terms were grading time (defined as PM for grading and AM for regrading), grader, and objective colour values (L*, a*, b*, Hue angle and Chroma). The random model was body number and date of slaughter. Then, a prediction of AUS-MEAT colour score from objective colour values was obtained from the REML analysis for AM and PM grading time. Model terms included for prediction were grading time, objective colour values (L*, a*, b*, Hue angle and Chroma), and date. To determine the differences between Dark Colour-Normal and Dark Colour-High pH in objective colour values at plant A, a REML analysis was conducted, fixed term was characteristic (either Dark Colour-Normal and Dark Colour-High pH). The random model was the date of slaughter, chiller, lot and grader.

4 Results

4.1 Time to from slaughter to grading analysis

Table 1 presents the summary of the number of carcasses included in the time to grading analysis.

Table 1. Summary of data collected between September 2017 and August 2018.

Total number of carcasses included in the study		**139,703**
By Plant	**A**	**68,223**
	B	40,968
	C	30,512
By Feedlot	G	6,754
	H	18,022
	I	18,775
	J	62,800
	K	6,072
	L	19,043
	M	8,237

4.1.1 Time from slaughter to grading

Between 84 and 89 % of carcasses were graded within 24 h post-slaughter in all processing plants (Table 2). Plant A had a minimum grading time of < 24 h post-slaughter for 85.1 % of carcasses and a maximum grading time of 96 h post-slaughter for 0.4 % of carcasses. Plant B had a minimum grading time of 24 h post-slaughter for 83.8 % of carcasses and a maximum grading time of 120 h from killing for 0.4 % of carcasses. Plant C had a minimum grading time of < 24 h post-slaughter for only 0.8 % of carcasses while 84.8 % of carcasses were graded at 24 h post-slaughter and 0.2 % of carcasses had a maximum grading time of 120 h from killing.

Table 2. Summary of data collected by grading time in each processing plant between September 2017 and August 2018.

Plant	**A**		**B**		**C**		
Grading Time	N	%	N	%	N	%	**Total**
< 24 h	58,090	85.1	0	0	243	0.8	**58,333**
1 day (24 h)	2,663	3.9	34,347	83.8	25,881	84.8	**62,891**
2 days (48 h)	6,673	9.8	210	0.5	338	1.1	**7,221**
3 days (72 h)	558	0.8	5,042	12.3	3,696	12.1	**9,296**
4 days (96 h)	239	0.4	1207	2.9	300	1.0	**1,746**
5 days (120 h)	0	0	162	0.4	54	0.2	**216**
Total	**68,233**	100	**41,968**	100	**30,527**	100	

Animals from feedlot I were normally graded < 24 h post slaughter at plant A while at plant B and C they were graded 24 h post-slaughter. Although animals from feedlot J were normally slaughtered at plant A and graded < 24 h post-slaughter, when animals from that feedlot were sent to plant C, they were normally graded 24 h post-slaughter (Table 3).

Table 3. Summary of data collected by grading time and by feedlot in each processing plant between September 2017 and August 2018.

Plant	**A**						**B**						**C**							
Producer	**I**		**J**		**K**		**H**		**I**		**L**		**G**		**I**		**J**		**M**	
Grading Time	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
< 24 h	15	100	52,357	84.3	5,718	94.6	0	0	0	0	0	0	0	0	72	0.5	0	0	171	2.1
1 day	0	0	2,314	3.7	319	5.3	11,866	65.8	3,903	100	18,578	97.6	6,684	99	14,002	94.2	664	100	4,531	55
2 days	0	0	6,668	10.7	5	0.1	0	0	0	0	210	1.1	70	1	268	1.8	0	0	0	0
3 days	0	0	0	0	0	0	4,887	27.1	0	0	155	0.8	0	0	461	3.1	0	0	3,235	39.3
4 days	0	0	239	0.4	0	0	1,107	6.1	0	0	100	0.5	0	0	0	0	0	0	300	3.6
5 days	0	0	0	0	0	0	162	0.9	0	0	0	0	0	0	54	0.4	0	0	0	0
total	15	100	62,136	100	6,072	100	18,022	100	3,903	100	19,043	100	6,754	100	14,857	100	664	100	8,237	100

4.1.2 Meat pH and colour

There was a high percentage of **Dark Colour-Normal pH** carcasses (10.6 %) in cattle slaughtered at plant A while the incidence of this characteristic was low in plant B (0.24 %) and zero in plant C. By contrast, the proportion of carcasses showing **Dark Colour-High pH** was similar among plants (2.8 % on average). There were no significant differences in the average pH at grading between plant A and B (5.56) but it was lower for plant C (5.52 SED ± 0.004; P < 0.001). There were significant differences in the average loin temperature at grading between plants, being higher for plant A, followed by plant B and C (10.0, 6.5, and 6.1 ± 0.05 ˚C) (Figure 1).

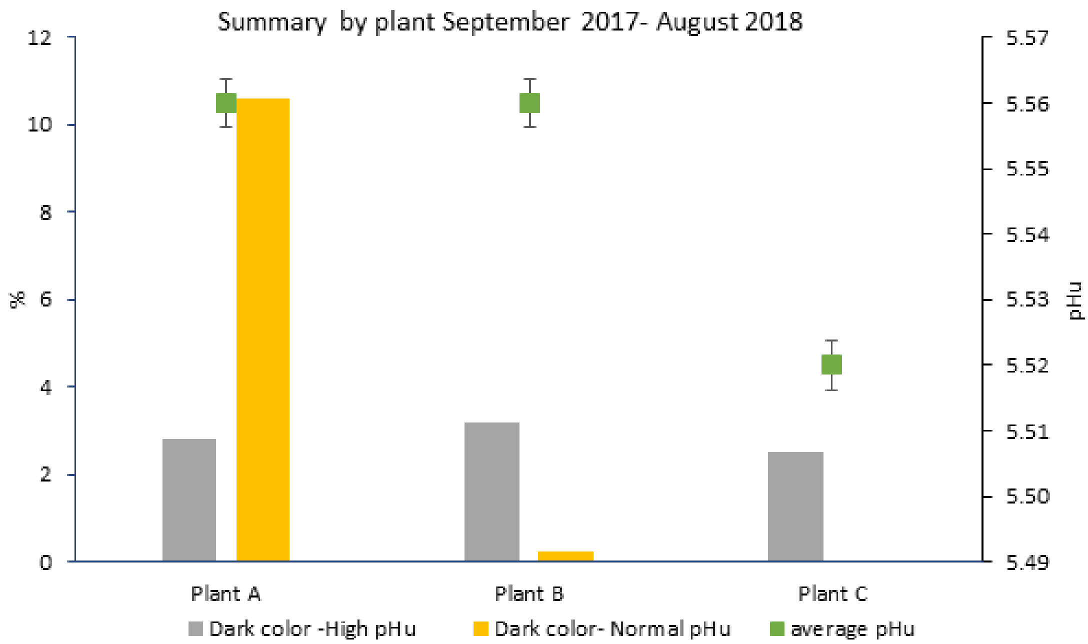

Figure 1. Percentage of Dark colour-high pHu, and Dark colour-normal pHu carcasses by plant from September 2017- August 2018. Green squares indicate average pH at grading ± SED.

There was a high percentage of **Dark Colour-Normal pH** carcasses in cattle from feedlot J and K (10.6 and 9.17 % respectively) followed by feedlot L (0.36 %) while the incidence of this characteristic was very low in the other feedlots. By contrast, the proportion of carcasses showing **Dark Colour-High pH** was higher for feedlot L (5.34 %) followed by feedlot G (3.01 %), and J (2.95 %), while the lowest was for feedlot K with 1.15 %. The incidence of **Normal Colour-High pH** carcasses was very low and only cattle from feedlot J processed at plant A had 0.1 % of carcasses with this characteristic (data not shown in tables and figures). Therefore, most of the carcasses showing high pH were associated with dark colour.

There were significant differences in the average pH at grading among feedlots, it was higher for feedlot G and L (5.56) followed by feedlot I and J (5.55) and it was lower for feedlots H, M and K (5.54 ± 0.003; P < 0.001; Figure 2). There were significant differences in the average loin temperature at grading among feedlots, it was higher for feedlots K, J, and G (8.1,7.8, and 7.7 ˚C) followed by feedlots H and I (7.6, and 7.5 ˚C) and it was lower for feedlots L and M (7.4 and 6.7 ± 0.04 ˚C; P < 0.001)

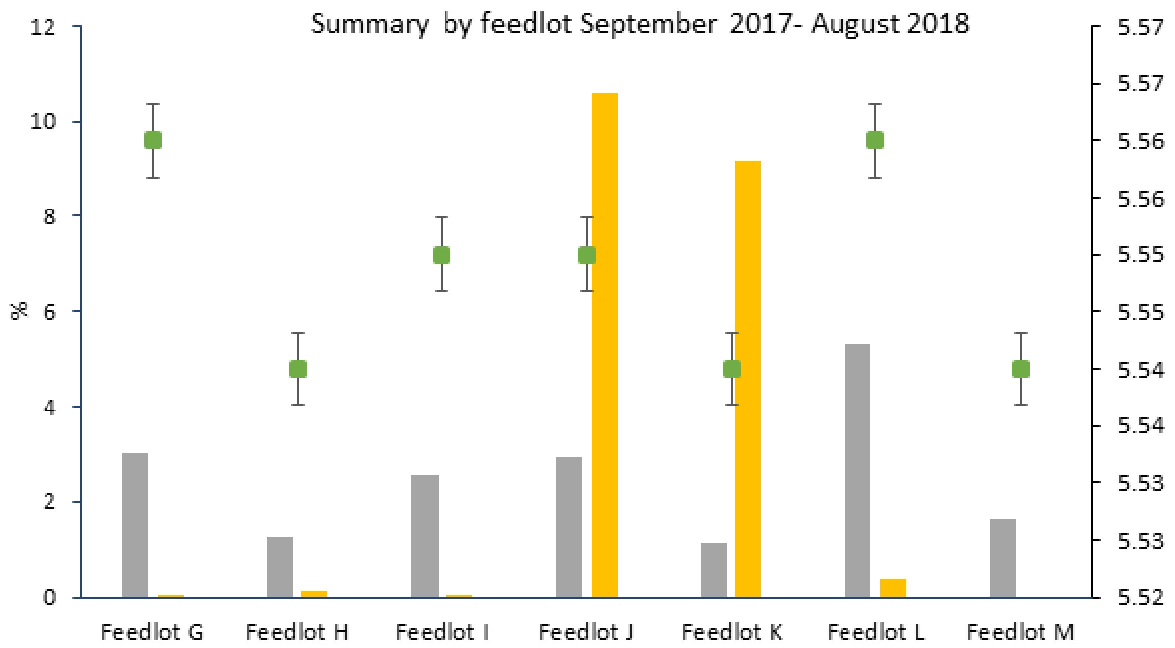

Figure 2. Percentage of Dark Colour-High pHu, and Dark Colour-Normal pHu carcasses by feedlot from September 2017- August 2018. Green squares indicate average pH at grading ± SED.

Although cattle from feedlot J were normally processed at plant A showing high percentages of **Dark Colour-High pH** and **Dark Colour-Normal pH** carcasses, those incidences were reduced when cattle from this feedlot was processed at plant C (Table 4 and 5). However, only 664 animals were killed at plant C compared to 62,136 killed at plant A. This indicates that numerical differences may confound any interpretation of the data.

4.1.3 Effect of time of grading on meat pH and colour

There was a high percentage of **Dark Colour-Normal pH** (11.6 %) when carcasses were graded < 24 h post-slaughter. Plant A graded at 13 h post-slaughter, on average, whereas plants B and C graded at 22 and 17 h, respectively. Hence the higher occurence of **Dark colour-Normal pH** for carcasses graded <24 h would have been skewed by plant A (see below).

By contrast, the incidence of carcasses showing **Dark Colour-High pH** was similar among grading times < 24 h, 1, and 2 days post-slaughter, while grading carcasses more than 3 days post-slaughter showed the lowest incidence of this characteristic. There were significant differences in the average pH at grading among grading times, it was higher < 24 h and 2 days post-slaughter (5.56) and it was lower at 120 h post-slaughter (5.50 ± 0.004; P <0.001) (Figure 3).

For plant A (Figure 4), carcasses graded within the first 24 h post-slaughter had a higher incidence of **Dark Colour-Normal pH** (25.5 %) compared to carcasses graded more than 1-day post-slaughter. The incidence of carcasses showing **Dark Colour-High pH** was higher 4 days post-slaughter (6.28 %) followed by 3- and 1-day post-slaughter. The lowest incidence was observed < 24 h post slaughter (2.6 %).

Table 4. Summary of carcass characteristics by processing plant and by feedlot between September 2017 and August 2018.

Plant	**A**						**B**						**C**							
Producer	**I**		**J**		**K**		**H**		**I**		**L**		**G**		**I**		**J**		**M**	
Carcass Characteristic	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Dark colour-High pHu	0	0	1,864	3.0	70	1.2	223	1.2	65	1.7	1,017	5.3	203	3.0	412	2.8	12	1.8	135	1.6
Dark colour- Normal pHu	1	6.67	6,711	10.8	559	9.2	25	0.1	4	0.1	69	0.4	1	0.02	0	0	0	0	0	0
Total Processed	**15**		**62,136**		**6,072**		**18,022**		**3,903**		**19,043**		**6,754**		**14,857**		**664**		**8,237**	

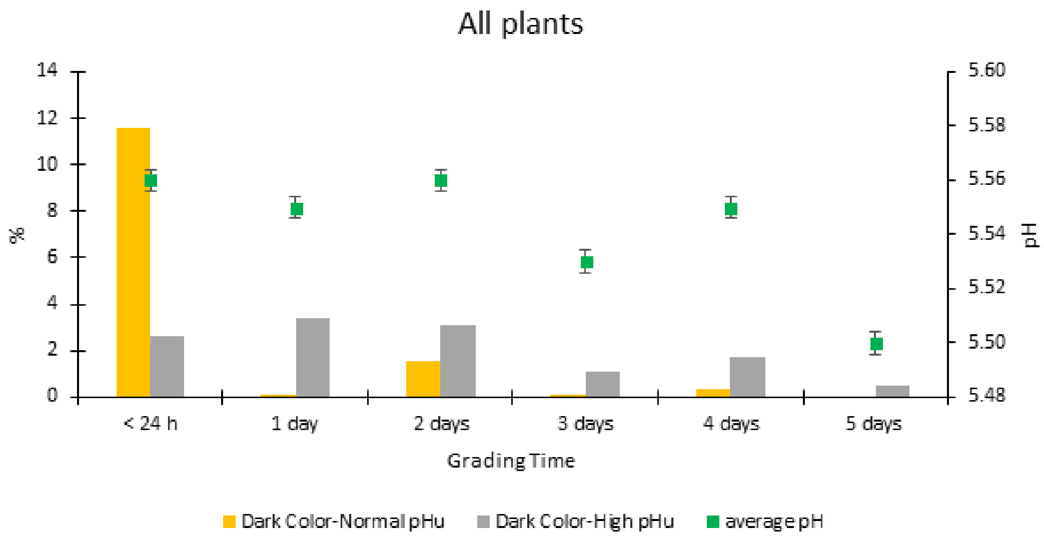

Figure 3. Proportion (%) Dark Colour-High pHu, and Dark Colour-Normal pHu carcasses by grading time from Sept 2017- August 2018. Green squares indicate average pH at grading ± SED.

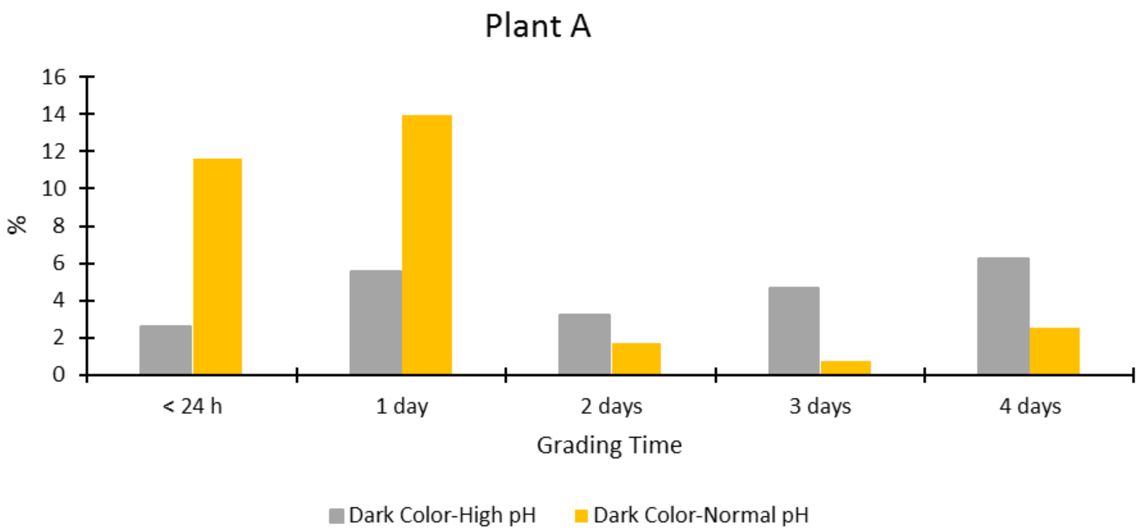

Figure 4. Proportion (%) Dark Colour-High pHu, and Dark Colour-normal pHu carcasses by grading time at plant A from Sept 2017- August 2018.

For plant B (Figure 5), by contrast, carcasses graded 24 h post-slaughter had a higher incidence of **Dark Colour-High pH** (3.62 %) compared to carcasses graded more than 1-day post-slaughter. The incidence of carcasses showing **Dark Colour-Normal pH** was very low at all grading times. For plant C (Figure 6), carcasses graded 24 h post-slaughter had a higher incidence of **Dark Colour-High pH** (2.79 %) compared to carcasses graded < 24 h and 2 days post-slaughter, while the incidence in carcasses graded more than 3 days post-slaughter was minimal. As mentioned previously, the incidence of carcasses showing Dark Colour-Normal pH was zero.

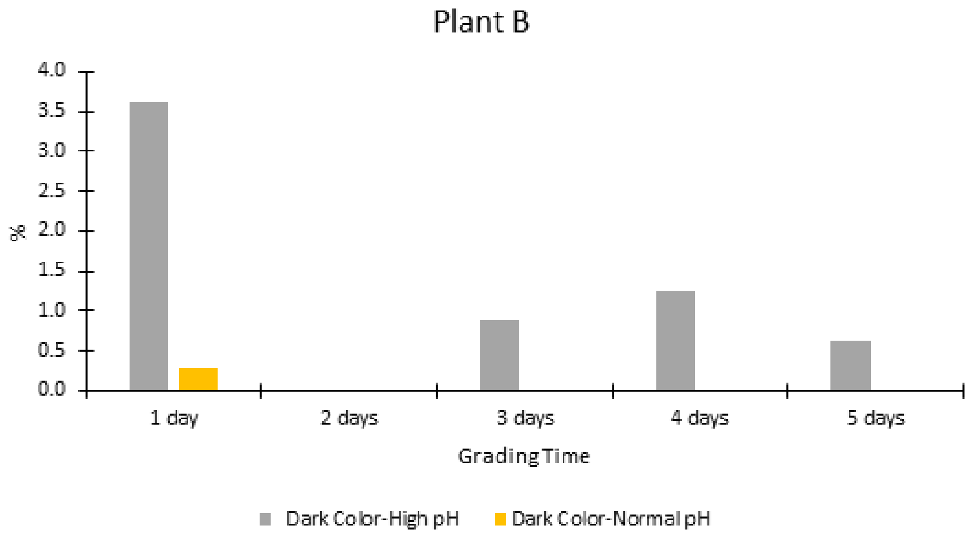

Figure 5. Proportion (%) Dark Colour-High pHu, and Dark colour-normal pHu carcasses by grading time at plant B from Sept 2017- August 2018.

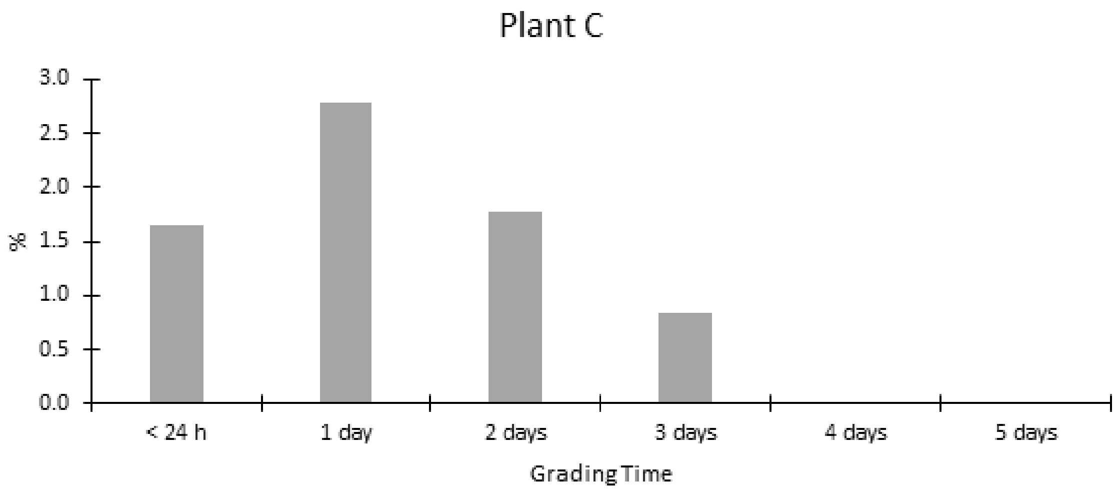

Figure 6. Proportion (%) Dark Colour-High pHu carcasses by grading time at plant C from Sept 2017- August 2018.

Table 5. Summary of carcass characteristics by time of grading for each feedlot and processing plant between September 2017 and August 2018

Time of Grading/Carcass Characteristic %		< 24 h		1 day		2 days		3 days		4 days		5 days	
Processing Plant	**Producer**	Dark colour-Normal pHu	Dark colour-High pHu	Dark colour-Normal pHu	Dark colour-High pHu	Dark colour-Normal pHu	Dark colour-High pHu	Dark colour-Normal pHu	Dark colour-High pHu	Dark colour-Normal pHu	Dark colour-High pHu	Dark colour-Normal pHu	Dark colour-High pHu
A	**I**	6.70	0	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	J	11.90	2.78	14.99	6.18	1.71	3.24	7.17	4.66	2.51	6.28	N/A	N/A
	K	9.31	1.14	6.88	1.43	0	0.0	N/A	N/A	N/A	N/A	N/A	N/A
B	**H**	N/A	N/A	0.21	1.39	N/A	N/A	0	0.88	0	1.27	0	0.62
	I	N/A	N/A	0.10	1.63	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	L	N/A	N/A	0.37	5.44	0	0	0	0.65	0	1.00	N/A	N/A
C	**G**	N/A	N/A	0.02	3.04	0	0	N/A	N/A	N/A	N/A	N/A	N/A
	I	0	2.78	0.0	2.84	0	2.41	0	1.52	N/A	N/A	0	0
	J	N/A	N/A	0.0	1.81	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	M	0	1.17	0.0	2.40	N/A	N/A	0	0.74	0	0	N/A	N/A

N/A: Not applicable

4.2 Grading and regrading analysis

Between September 2017 and August 2018, a total of 2,454 carcasses were assessed for grading and pH-temperature decline; 1,357 carcasses from plant A, 897 carcasses from plant B, and 200 carcasses from plant C. Of those 1,357 carcasses from plant A, 1,181 carcasses were assessed for re-grading, all those carcasses belong feedlot J. Objective colour determination using NIX was obtained from 1,828 carcasses; 1,041 carcasses from plant A, 587 carcasses from plant B, and 200 carcasses from plant C. Data collected in July and August 2018 from plant A was focused on grading and regrading of **Dark Colour- High pH** carcasses only.

4.2.1 Temperature@pH6

There were no differences in Temp@pH6 between plants ($P < 0.05$ for all; Figure 7), however there were differences between plants in the 2nd temperature and pH (Table 6).

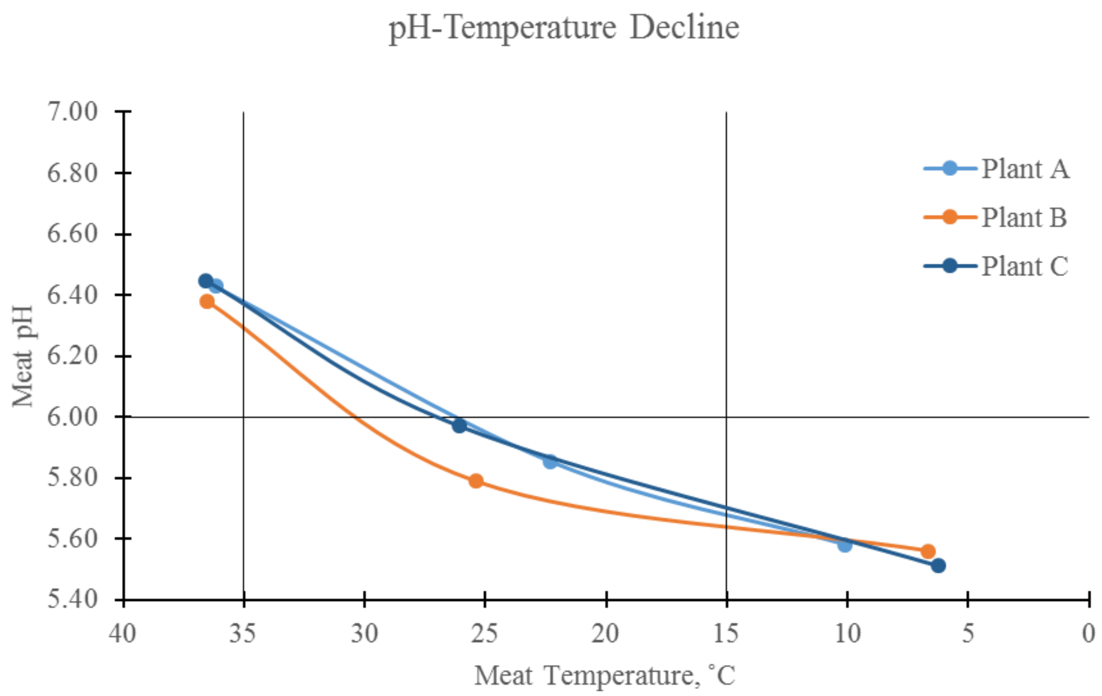

Figure 7. pH and temperature window observed in all processing plants included in this study.

There were no differences in grading pH between plants, however significant differences were observed in the average loin temperature at grading, being higher for plant A, followed by plant B and C (10.0, 6.7, and 6.3 ± 0.5 ˚C). Plant A had higher incidences of cold shortening than plant B and C, and incidences of heat shortening were similar between plant A and B (Table 6).

The incidences of **Dark Colour-High pH**, and **Dark Colour-Normal pH** carcasses were not associated with the Temp@pH6 in plant B and C ($P > 0.05$). For plant A, it was demonstrated that the incidences of **Dark Colour-High pH**, and **Dark Colour-Normal pH** carcasses were inversely related to the Temp@pH6 ($P < 0.001$) and associated to cold shortening since Temp@pH6 < 12 ˚C was predicted to have high incidences of **Dark Colour-High pH** carcases (Figure 8; Table 7).

Table 6. Summary of data obtained to calculate Temp@pH6 and summary of carcass characteristics at grading (N= 2,454)

Item	Plant A	Plant B	Plant C	SED	P-value
1st pH	6.43	6.38	6.45	0.048	0.213
1st Temp,˚C	36.4	36.6	36.6	0.895	0.939
2nd pH	5.90	5.79	5.97	0.066	0.017
2nd Temp,˚C	22	25.4	26.1	1.265	0.001
Temp@pH6, ˚C	22.6	23.8	22.7	1.676	0.524
Grading pH	5.33	5.56	5.51	0.082	0.091
Grading Temp,˚C	10	6.7	6.3	0.5	<0.001
Heat Shortening, %	2.6	3	1		
Cold Shortening, %	4.2	1.1	0.5		
Dark Color-Normal pH, %	17.6	0	0		
Dark Color-High pH, %	7.2	2.5	2		

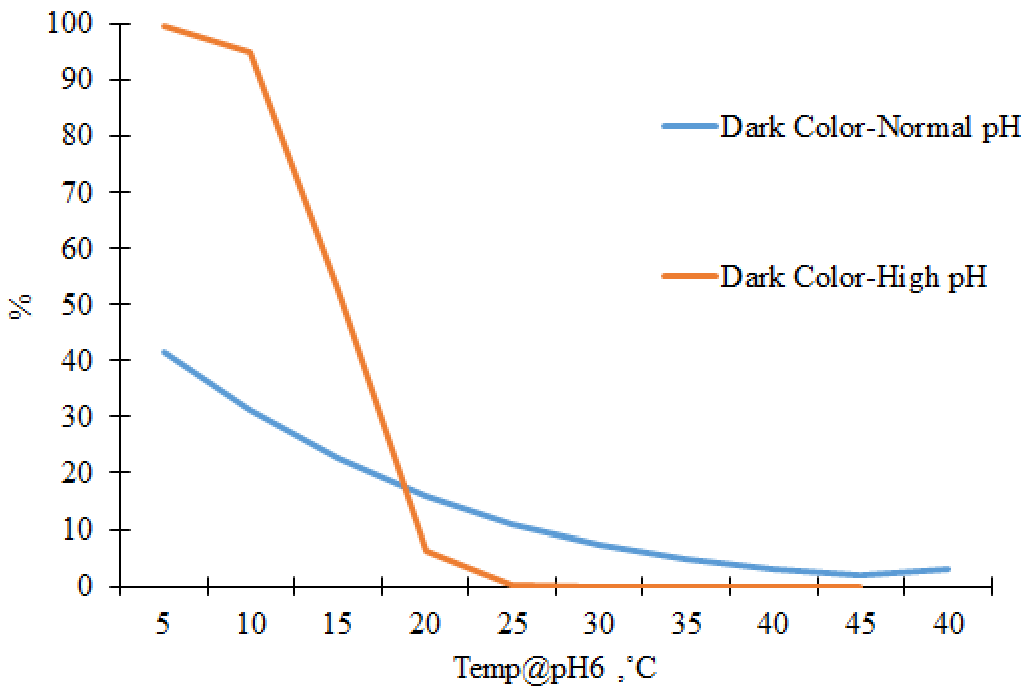

Figure 8. Predicted Dark Colour- High pH and Dark Colour- Normal pH percentages at grading from Temp@pH6. For the purposes of the predicted values the following parameters were used in the model = Constant, Temp@pH6, MSA marbling, P8Fat, and Total HSCW. Random factors were Day of Kill, Chiller, Lot, and Grader.

4.2.2 Effect of carcass characteristics on meat pH and colour

For plant A, carcass characteristics were associated with the incidences of **Dark Colour-Normal pH** meat at grading. Prediction model indicated that reduced rump fat thickness, reduced marbling scores and lighter carcasses all were associated with higher percentages of **Dark Colour-Normal pH** meat (P

< 0.05; Table 14, Figure 9). No effects of carcass characteristics were observed on the incidences of **Dark Colour-Normal pH** and **Dark Colour-High pH** meat at plant B and C for carcasses included in grading analysis.

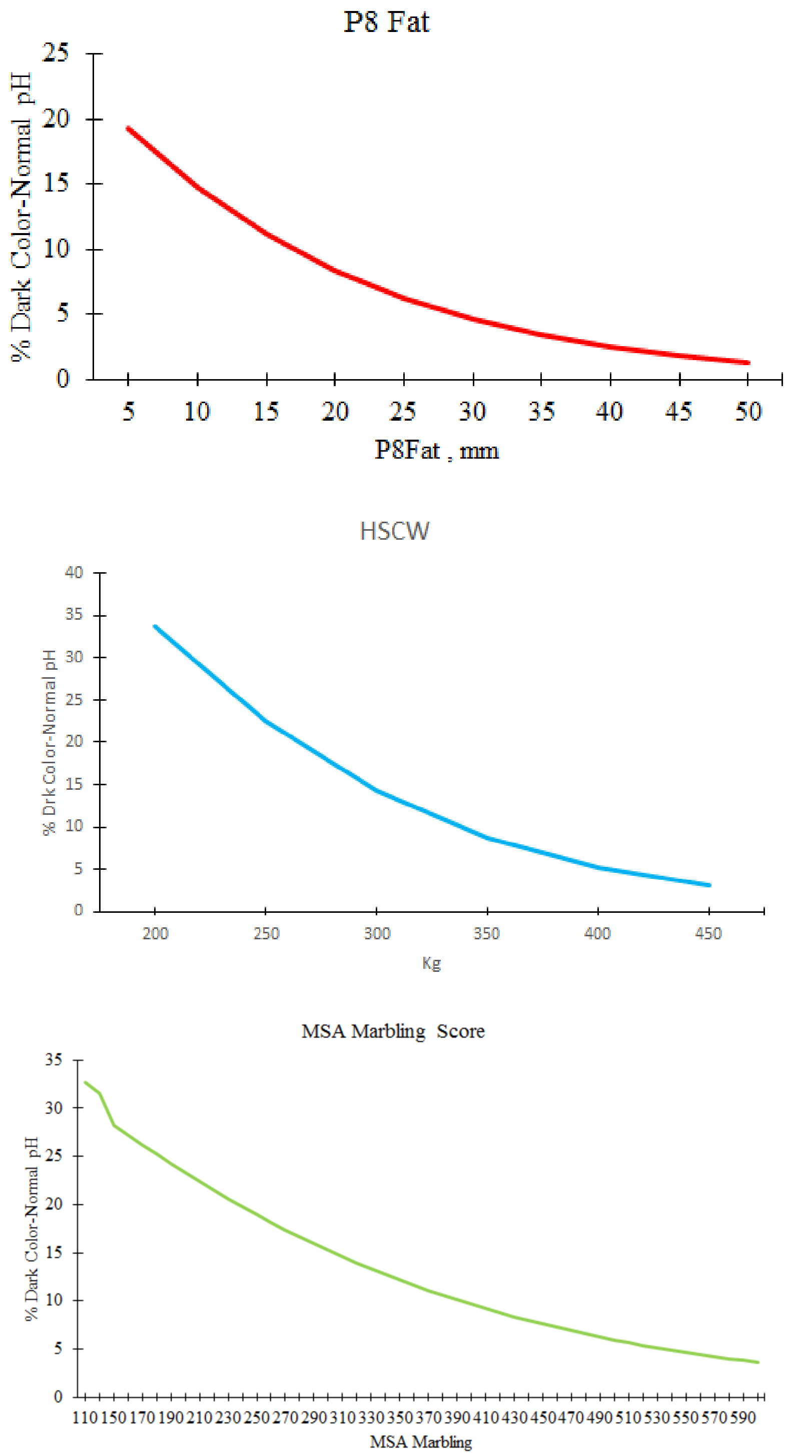

Figure 9. Effect of (a) P8 Fat, (b) MSA Marbling Score and (c) HSCW on the percentage of Dark Colour-Normal pH meat. For the purposes of the predicted values the following parameters were used in the model = Day of Kill, Chiller, Lot, Grader, MSA Marbling, P8Fat, Temp@ph6, and Total HSCW

Table 7. Effect on the percentage of Dark Colour-Normal pH meat of MSA marbling, P8 Fat, Temp@pH6, and HSCW at plant A.

Fixed term	Coefficient ±SE	Prob
Constant	-1.910 ± 0.2505	
MSA Marbling	-0.0052 ± 0.00188	0.005
P8 Fat	-0.0642 ± 0.02079	0.002
Total HSCW	-0.0111 ± 0.00313	< 0.001
Temp@pH6	-0.0877 ± 0.01892	<0.001

4.2.3 Effect of time from slaughter to grading and regrading on meat pH and colour

Time from slaughter to grading was associated with the percentage of carcasses showing AUS-MEAT colour score >3 and Dark Colour-Normal pH meat (P<0.001). Prediction model indicated that longer time from slaughter to grading was associated with lower percentages of dark colour meat (Figure 10) and lower percentages of **Dark Colour-Normal pH** carcasses (Figure 11). No association between time from slaughter to grading was observed on the percentage of high pH carcasses and **Dark Colour-High pH** carcasses (Table 8).

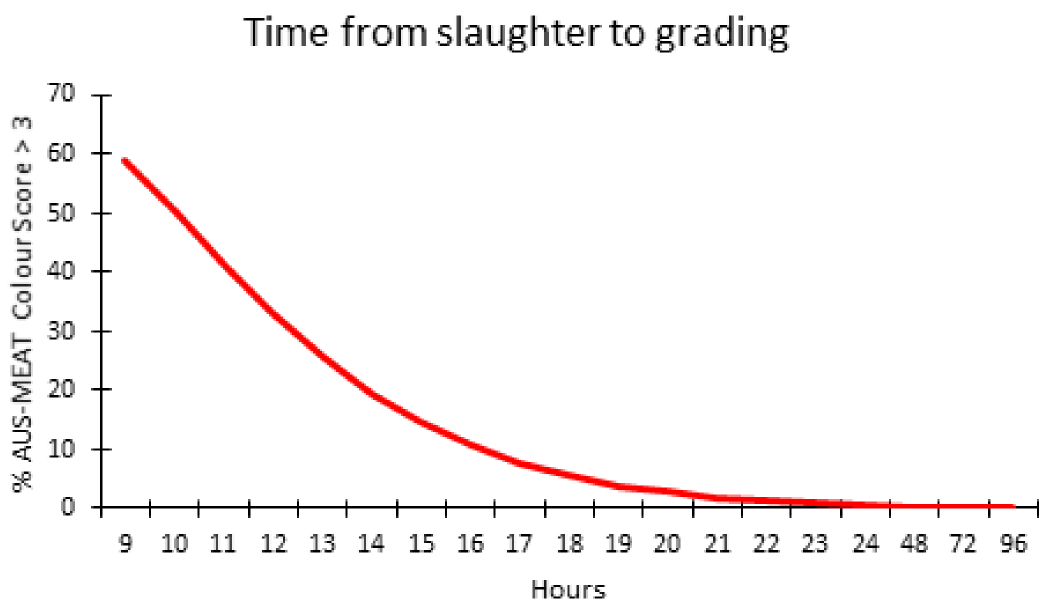

Figure 10. Effect of time from slaughter to grading on the percentage of AUS-MEAT Colour score >3. For the purposes of the predicted values the following parameters were used in the model = Day of slaughter, and time from slaughter to grading.

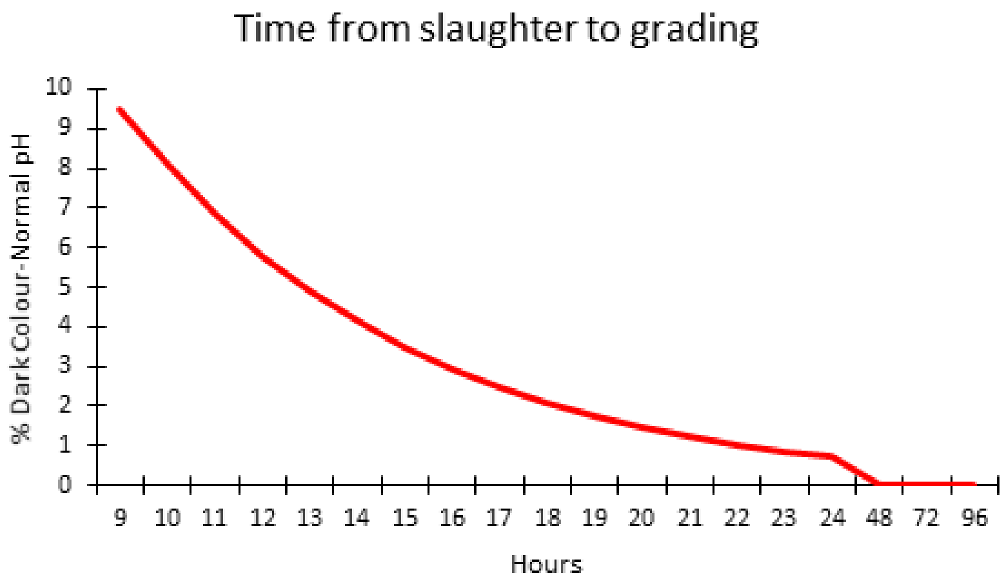

Figure 11. Effect of time from slaughter to grading on the percentage of Dark Colour-Normal pH meat. For the purposes of the predicted values the following parameters were used in the model = Day of slaughter, and time from slaughter to grading.

Table 8. Effect on the percentage of AUS-MEAT Colour score >3 and Dark Colour-Normal pH meat of time from slaughter to grading

Carcass Characteristic	**AUS-MEAT colour score >3**		**Dark Colour-Normal pH**	
Fixed term	Coefficient ±SE	Prob	Coefficient ±SE	Prob
Constant	3.586 ± 1.1148		-0.5607 ± 0.9197	
Time from slaughter to grading	-0.3573 ± 0.0685	<0.001	-0.1779± 0.0544	0.002

4.2.4 Grading and regrading time

Grading at plant A was normally conducted between 19:30 and 2:30 AM, while at plant B it was conducted between 6:00 AM and 10:00 AM, and in plant C between 2:00 AM and 6:00 AM. Data presented in Table 9 shows the mean, min and max grading time, and regrading time, for each processing plant. Grading at plant A was conducted by three graders and regrading was conducted with the help of a 4th different grader, regrading was done between 4:00 and 5:00 AM. Grading at plant B and C was conducted by five and three different graders, respectively.

Table 9. Summary of grading and regrading time for each processing plant

Plant	A			B	C
Item	Time from kill to grading (h)	Time from kill to regrading (h)	Time from grading to regrading (h)	Time from kill to grading (h)	Time from kill to grading (h)
Mean	12.6	21.1	8.6	21.9	17.2
Minimum	10.2	14.9	2.9	18.6	12.8
Maximum	15.9	24.0	10.1	25.3	19.7
S.D	1.15	1.15	0.79	1.93	2.51

4.2.5 Objective colour analysis

There were differences in objective colour values (L*, a*, b*) between plants at grading. Therefore, data were analysed for each plant separately. It was demonstrated that CIE Lab*, Hue and Chroma values were inversely related to the darkness of the meat surface. For plant A, there were differences in all objective colour values between AUS-MEAT colour scores; ($P < 0.001$ for all). For plant B, there were differences in L* and a* between AUS-MEAT colour scores; ($P < 0.05$ for all). For plant C, no differences were observed in Hue angle between AUS-MEAT colour scores (Table 10).

In general, no differences between graders were observed at grading (data not shown). However, in plant A, differences were observed between graders in L* value (grader 1= 36.65, grader 2 =36.13, grader 3=34.63, and grader 4= 36.09 ± 0.8623; $P = 0.003$). It needs to be acknowledged that grader 3 only graded carcasses during the last month of the study, however, this grader was responsible of regrading. At regrading, there were differences in all objective colour values between AUS-MEAT colour scores; ($P < 0.001$ for all) and, as grading, there was an inverse relationship between the degree of darkness and the objective colour values (Table 11).

Table 10. Objective colour values and AUS-MEAT colour scores at grading in all plants*

Colour Value/Plant	L* (Lightness)			a*(Redness)			b* (Yellowness)			Chroma			Hue angle		
AUS-MEAT Colour Score	**Plant A**	**Plant B**	**Plant C**	**Plant A**	**Plant B**	**Plant C**	**Plant A**	**Plant B**	**Plant C**	**Plant A**	**Plant B**	**Plant C**	**Plant A**	**Plant B**	**Plant C**
1	40.03	40.94	39.32	20.62	25.63	21.99	13.17	15.56	13.90	24.47	26.56	26.03	32.67	31.13	32.38
2	38.70	39.66	38.69	20.8	25.78	23.57	12.88	14.86	14.83	24.53	26.67	27.82	31.87	31.49	32.37
3	36.79	38.62	37.06	19.51	25.27	22.3	11.84	15.38	13.80	22.82	27.10	26.26	31.30	31.38	31.75
4	34.73	38.48	38.99	17.51	24.68	21.17	10.51	14.18	14.46	20.43	27.03	25.72	31.10	31.04	34.01
5	33.05	38.97	40.78	16.14	24.12	24.4	9.52	13.58	15.31	18.73	28.6	28.79	30.78	31.64	32.44
6	31.96	N/A	N/A	14.95	N/A	N/A	8.77	N/A	N/A	17.32	N/A	N/A	30.69	N/A	N/A
SED	0.315	0.733	1.684	0.311	0.634	1.685	0.190	1.862	1.108	0.363	1.137	1.951	0.199	0.465	0.921
P-Value	< 0.001	<0.001	0.007	<0.001	0.052	0.005	<0.001	0.781	0.007	< 0.001	0.517	0.006	<0.001	0.313	0.237

*Objective colour determination using NIX was obtained from 1,828 carcasses; 1,041 carcasses from plant A, 587 carcasses from plant B, and 200 carcasses from plant C
N/A; not applicable.

Table 11. Objective colour values and AUS-MEAT colour scores at grading and regrading† at plant A (n= 1,041)

Colour Value/Time	**L* (Lightness)**		**a*(Redness)**		**b* (Yellowness)**		**Chroma**		**Hue angle**	
Meat Colour	Grading	Regrading	Grading	Regrading	Grading	Regrading	Grading	Regrading	Grading	Regrading
1	40.03	43.94	20.62	21.03	13.17	14.61	24.47	25.59	32.67	34.68
2	38.70	42.24	20.80	21.99	12.88	14.95	24.53	26.61	31.87	34.19
3	36.79	40.88	19.51	22.39	11.84	14.75	22.82	26.85	31.30	33.42
4	34.73	39.75	17.51	21.82	10.51	14.22	20.43	26.04	31.10	32.06
5	33.05	37.48	16.14	19.32	9.52	12.38	18.73	22.99	30.78	32.59
6	31.96	34.25	14.95	15.22	8.77	9.88	17.32	18.01	30.69	33.62
7*	N/A	29.55	N/A	12.41	N/A	8.13	N/A	14.88	N/A	33.19
SED	0.315	1.041	0.311	0.971	0.190	0.569	0.363	1.092	0.199	0.779
P-Value	< 0.001	<0.001	<0.001	<0.001	<0.001	<0.001	< 0.001	<0.001	<0.001	<0.001

*One observation
† P-value difference grading and regrading (P<0.001). N/A; not applicable.

Objective colour values were different between grading and regrading, being higher at regrading ($P < 0.001$ for all; Table 11). However, differences determined by objective colour determination were not associated with differences reported by the grader. At regrading, the grader consistently reported darker meat colour (AUS-MEAT colour score > 3) while objective colour determination reported lighter (higher L*) and redder (higher a*) meat than at grading (Figure 12 and 13).

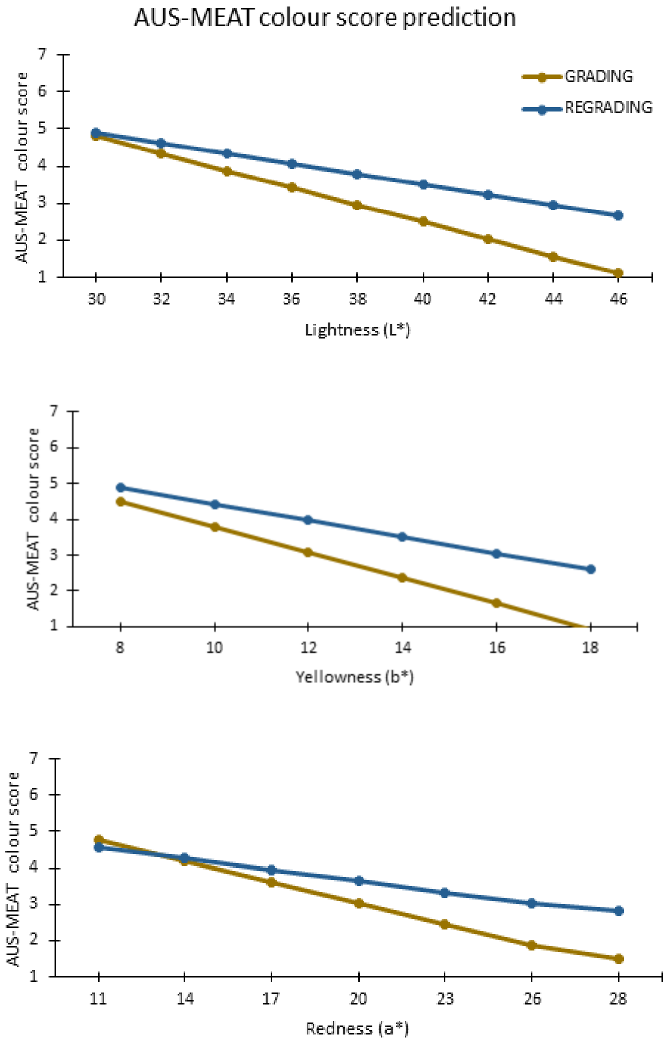

Figure 12. Predicted AUS-MEAT colour score at grading and regrading from objective colour values Lightness, Redness, and Yellowness. For the purposes of the predicted values the following parameters were used in the model = Constant, Grading Time, objective colour values (L*, a*, b*), and date.

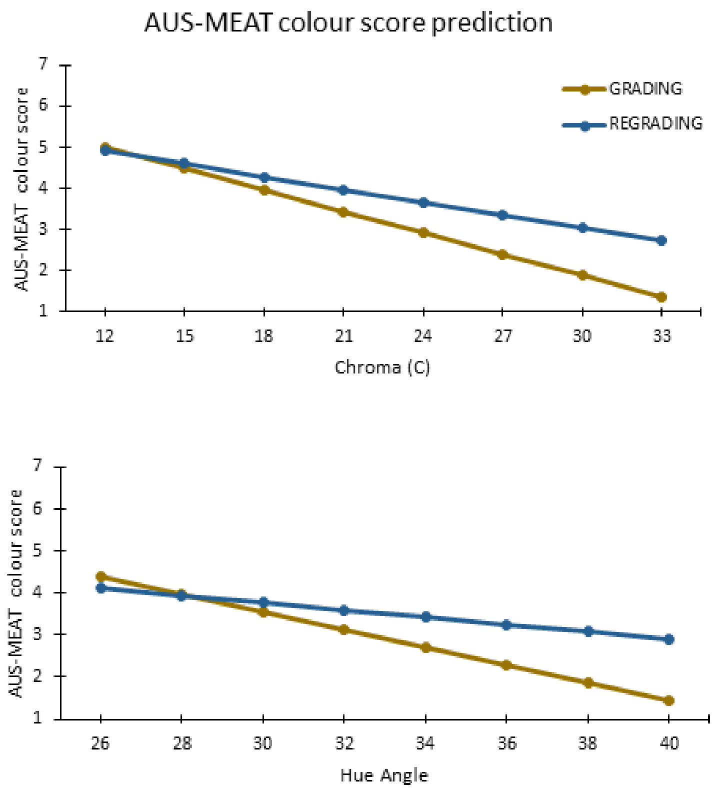

Figure 13. Predicted AUS-MEAT colour score at grading and regrading from objective colour values Chroma and Hue angle. For the purposes of the predicted values the following parameters were used in the model = Constant, Grading Time, objective colour values (Chroma and Hue angle), and date.

There were significant differences in objective colour values between carcass characteristics such that Dark Colour-Normal pH carcasses had higher values for L*, a*, b*, and Chroma than Dark Colour- High pH carcasses (P<0.001; Table 12).

Table 12. Objective colour values for each carcass characteristic at grading at plant A.

Characteristic	L* (Lightness)	a* (Redness)	b* (Yellowness)	Chroma	Hue Angle	pH	t°
Dark Colour-Normal pH	34.75	17.57	10.39	20.44	30.83	5.58	9.7
Dark Colour-High pH	32.65	15.05	8.76	17.45	30.74	6.16	9.7
SED	0.328	0.341	0.226	0.406	0.224	0.021	0.190
P-value	<0.001	<0.001	<0.001	<0.001	0.709	<0.001	0.890

4.2.6 Effect of grading and regrading on meat pH and colour

At grading, the incidence of dark colour carcasses (AUS-MEAT colour score >3) was 30 %, and the average pH of carcasses showing AUS-MEAT colour score 4 was 5.60. This indicates that there was a high proportion of carcasses showing AUS-MEAT colour score 4 but normal pH at grading (91 %).
At regarding, the average pH of carcasses showing AUS-MEAT colour score 4 was 5.51 indicating a reduction in pH from grading. However, there was an increased proportion of carcasses showing meat colour score 4 and normal pH at regrading (95.2 %) compared to grading. Indeed, there was an increase in 11.7 % in the incidence of dark colour carcasses at regrading (AUS-MEAT colour score > 3; 30.1 vs 41.8 % at grading and regrading, respectively; Table 13 and 14, Figure 14).

When comparing graded and regraded carcasses (n= 1181), 77.7 % of graded carcasses showed a reduction of pH when regraded at 21.1 h post-slaughter independently of their pH grading (Table 15). The mean pH at grading was 5.59 ± 0.195 (min 5.36, max 6.79) and the mean pH at regrading was 5.52 ± 0.186 (min 5.29, max 6.81, t = 7.95 d.f= 2080 , P < 0.001).

For meat colour, 45.6 % of all graded carcasses showed an increment in AUS-MEAT colour score when regraded at 21.1 h post-slaughter independently of their colour score at grading (Table 15). The mean colour score at grading was 3.04 ± 1.32 (min 1 max 6) and the mean colour score at regrading was 3.4 ± 0.918 (min 1, max 7, t =-7.19 d.f=1845.65. P < 0.001). This result is highly indicative of differences between graders in their scoring for colour, as demonstrated by the discrepancy between colour measurements and colour scores between graders, shown in Figures 12 and 13.

When regrading non-compliant carcasses (**Dark Colour-High pH**), 81.8 % of those carcasses showed a reduction in pH and 32.3 % and became complaint in pH, and 7.07 % became compliant in colour (AUS-MEAT colour score ≤ 3). When regrading **Dark Colour-Normal pH** carcasses, 57.8 % of them showed a reduction in AUS-MEAT colour score at regrading and 34.8 % showed colour score ≤ 3. Nevertheless, when compliant (normal pH and normal colour) carcasses were regraded, 17.5 % and 61.6 % of them showed an increment in pH and AUS-MEAT colour score, respectively. Indeed, 29.3 % had meat colour darker than AUS-MEAT 3 (Figure 14, Table 15).

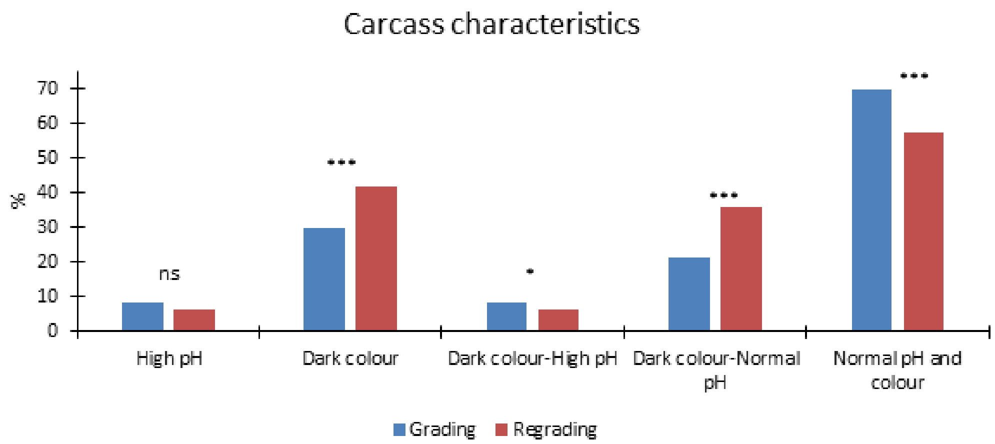

Figure 14. Change in carcass characteristics from grading to regrading in plant A. *** = P<0.001, *=P<0.05, ns= P>0.05

Table 13. Average pHu (s.d) and proportion (%) of dark colour (MSA colour score > 3), high pHu (pHu > 5.70), and carcasses showing both characteristics at grading

GRADING 12.6 h ± 1.15 min													
AUS-MEAT colour score	**N**	**%**	**mean pHu (s.d)**	**Dark colour %**	**Number of carcasses**	**High pHu %**	**Number of carcasses**	**Dark colour-High pHu %**	**Number of carcasses**	**Dark colour-Normal pHu %**	**Number of carcasses**	**High pH-Normal colour %**	**Number of carcasses**
1B	0	0	0	0	0	0	0	0	0	0	0	0	0
1C	109	9.2	5.51 (0.039)	0	0	0	0	0	0	0	0	0	0
2	340	28.8	5.53 (0.049)	0	0	0	0	0	0	0	0	0	0
3	377	31.9	5.54 (0.067)	0	0	0.3	1	0	0	0	0	0.3	1
4	193	16.3	5.60 (0.164)	100	193	8.8	17	8.8	17	91.2	176	0	0
5	76	6.4	5.76 (0.268)	100	76	39.5	30	39.5	30	60.5	46	0	0
6	86	7.3	5.98 (0.375)	100	86	60.5	52	60.5	52	39.5	34	0	0
7	0	0	N/A	0	0	0	0	0	0	0	0	0	0
Total	1181	100	5.59 (0.192)	30.1	355	8.5	100	8.4	99	21.7	256	0.1	1

N/A: Not applicable.
* Data shown in table 13, 14 and 15 were generated on the same carcasses at the same plant (A) but by different graders.

Table 14. Average pHu (s.d) and proportion (%) of dark colour (MSA colour score > 3), high pHu (pH> 5.70), and carcasses showing both characteristics at regrading*

REGRADING 21.1 h ± 1.15 min													
AUS-MEAT colour score	**N**	**%**	**mean pH (s.d)**	**Dark colour %**	**Number of carcasses**	**High pH %**	**Number of carcasses**	**Dark colour-High pH %**	**Number of carcasses**	**Dark colour-Normal pH %**	**Number of carcasses**	**High pH-Normal colour %**	**Number of carcasses**
1B	1	0.1	5.5 (0)	0	0	0	0	0	0	0	0	0	0
1C	11	0.9	5.41 (0.052)	0	0	0	0	0	0	0	0	0	0
2	160	13.6	5.44 (0.057)	0	0	0	0	0	0	0	0	0	0
3	507	43.3	5.48 (0.070)	0	0	1.2	6	0	0	0	0	1.2	6
4	394	33.6	5.51 (0.114)	100	394	4.8	19	4.8	19	95.2	375	0	0
5	69	6	5.73 (0.292)	100	71	37.7	26	37.7	26	62.3	43	0	0
6	28	2.4	6.27 (0.333)	100	28	92.9	26	92.9	26	7.1	2	0	0
7	1	0.1	6.35 (0)	100	1	100	1	100	1	0	0	0	0
Total	1181	100	5.52 (0.185)	41.8	494	6.6	78	6.4	76	35.6	420	0.5	6

* Data shown in table 13, 14 and 15 were generated on the same carcasses at the same plant (A) but by different graders.

Table 15. Percentage of carcasses showing an increase or decrease of pH and AUSMEAT colour score between grading and regrading*

Characteristic	N (%) at grading	N (%) at regrading	Decrease pH at Re-grading (%)	Increase pH at re-grading (%)	Decrease AUSMEAT colour score at re-grading (%)	Increase AUSMEAT colour score at re-grading (%)	Become Compliant pH if non-compliant at grading (%)	Become Non-compliant pH if compliant at grading (%)	Become normal in colour if dark at grading (%)	Become dark if normal at grading (%)
Total	1,181 (100)	1,181 (100)	77.7	18.5	20	45.6	2.7	1.3	8.1	20.6
High pH	100 (8.5)	78 (6.6)	82.0	13	N/A	N/A	32	N/A	N/A	N/A
Dark colour	355 (30.1)	494 (41.8)	N/A	N/A	54.6	7.0	N/A	N/A	27.0	N/A
Dark colour-High pH	99 (8.4)	76 (6.4)	81.8	13.1	51.7	7.9	32.3	N/A	7.07	N/A
Dark colour-Normal pH	256 (21.6)	420 (35.6)	71.9	24.2	57.8	7.0	N/A	3.5	34.8	N/A
Normal colour-High pH	1 (0.1)	6 (0.5)	0	0	0	100	0	N/A	N/A	100
Normal pH and colour	825 (69.9)	679 (57.5)	79	17.5	4.9	61.6	N/A	0.73	N/A	29.3

N/A: not applicable

* Data shown in table 13, 14 and 15 were generated on the same carcasses at the same plant (A) but by different graders.

5 Potential economic benefits of re-grading of beef carcasses which are non-compliant for pH at grading

5.1 Introduction

High ultimate pH (pHu) in beef carcasses is a considerable economic cost to the beef industry (Hughes et al., 2017). In meat science around the world, the phenomenon of high pHu is usually labelled as 'dark-cutting' or dark, firm, dry (DFD), due the association between high pHu and meat colour. In this report, we will use the terms, high pH when we are referring to carcasses non-compliant for pH at grading (pH >5.7; MSA specification) and the term 'dark colour', when we are referring to carcasses non-compliant to company specifications for colour (AUSMEAT colour score >3). Usually the incidence of high pH in beef carcasses of pasture-fed cattle is about 10-12%, while in grain-fed cattle it is about 1% (Warner et al., 1988). Recent data suggest that some abattoirs have a much higher incidence of high pH in grain-fed cattle (Hughes et al., 2017). In addition, Hughes et al. (2014) investigated the colour and pH of beef carcasses graded over the range of 13-32 hrs post-mortem and showed that if the time to grading was 14 hrs, the incidence of carcasses with colour scores >3 was 8%, whereas if graded at 31 hrs, this incidence dropped to 3%. Previous studies have demonstrated that the LT muscle of beef carcasses chilled by traditional methods, does not reach ultimate pH until at least 18-24 h post slaughter (Fabiansson et al., 1984; Murray, 1989). Previous reports have estimated that a high pH carcass decreases in value by $50-1,000 per carcass (Hughes et al., 2017).

The aim of this study was to document the potential economic benefits of re-grading of beef carcasses which were non-compliant for pH at grading

5.2 Materials and Method

A commercial beef abattoir supplied data on the price per kg for all the major cuts for MSA, and non-MSA, cuts for a 100 day grain-fed beef carcass (see Table 1). An average hot carcass weight of 280 kg was used. The proportions of the carcass that each cut represents comes from AUSMEAT industry handbook which is an indication of an average proportion across industry for those cuts (Table 1). An incidence of high pH at grading of 8.4% was used and an increase in pH compliance at re-grading of 32.3% (Gonzalez Rivas et al., 2019). This was from the regrading data of 1181 beef carcasses graded at ~12 hrs post-slaughter and re-grading ~23 hrs post-slaughter.

For the calculations, a daily carcass throughput of 1,000 was used. Furthermore, it was observed, during the re-grading process, that there would not be any increase in chiller capacity required as the carcasses were held for a period when capacity was available. Thus, the cost of re-grading was estimated to only involve the extra time required by MSA graders. This was estimated to be a 10 min set time per day, to find the carcasses, and 1 min per carcass to re-measure and enter the new pH. MSA graders time was estimated to be $65/hr. Using a standard HCWT of 280 kg, which is a standard weight for a 100 day grain-fed carcass, calculations and expressed in graphs.

Table 16. Pricing for the cuts from a beef carcass, for 100 Day Grain Fed MSA and non-MSA graded beef. The % of the carcass for each cut (AUSMEAT handbook, 2008) is also indicated.

PRODUCT	MSA	non-MSA	% of carcass
EYE ROUND	$ 8.70	$ 8.50	1.4
OYSTER BLADE	$ 10.80	$ 9.50	1.7
NE Brisket	$ 7.00	$ 7.00	3.3
PEDO Brisket	$ 8.70	$ 8.00	3.8
SHORT RIBS	$ 16.00	$ 15.00	2
BOLAR BLADES	$ 8.10	$ 7.20	5.5
CHUCK ROLLS	$ 8.70	$ 8.50	4.8
TRI- TIP	$ 10.00	$ 9.00	1
TOPSIDE	$ 8.60	$ 8.40	6.2
OUTSIDE FLAT	$ 7.50	$ 7.20	3.8
KNUCKLE	$ 7.65	$ 7.40	3.3
FLANK STEAK	$ 12.00	$ 11.50	3.7
STRIPLOIN	$ 15.50	$ 12.00	4.4
CUBE ROLL	$ 25.50	$ 22.00	2.8
TENDERLOIN	$ 31.00	$ 26.00	1.6
RUMP	$ 10.00	$ 8.50	3.8

5.3 Results

Assuming an HCWT of 280 kg and the $/kg and % of carcass for each cut in Table 1, the value of the cuts in a normal carcass was $1,662.29. For a high pH carcass which produced non-MSA graded cuts, the same cuts were valued at $1499.85. Hence the loss per carcass due to high pH non-compliance is $162.44 per carcass for the processor.

Figure 1 shows the value of 1,000 carcasses at grading and depending on the incidence of high pH at grading, the re-calculated value at re-grading. The calculations assume that 32.3% of carcasses non-compliant for pH at grading, are compliant at re-grading. Note that the % carcasses becoming compliant for pH at regrading, is similar to the % carcasses becoming complaint about colour at a later time of grading (Hughes et al., 2014), hence verifying the validity of this data. In Figure 1, it is evident that at a low incidence of non-compliance, the increase in value of the carcasses at regrading is negligible. For a 10% incidence of high pH at grading, the increase in value between grading and re-grading is $5,247 per day which is a significant increase.

Figure 2 shows the net benefit, after adjusting for the cost of labour to regrade. For these calculations, the assumptions are shown in figure heading. Over 1,000 carcasses, if the % high pH at grading was 10%, the net benefit is $5,129.

The only data available was for grading occurring at ~12 hrs, and regrading at ~23 hrs. If the grading occurs later than 12 hrs, the benefit would decrease, as the % carcasses reaching pH compliance at regrading would be lower. Conversely, if the grading occurs before 12 hrs, the benefit would be higher, as more carcasses would reach compliance at regrading.

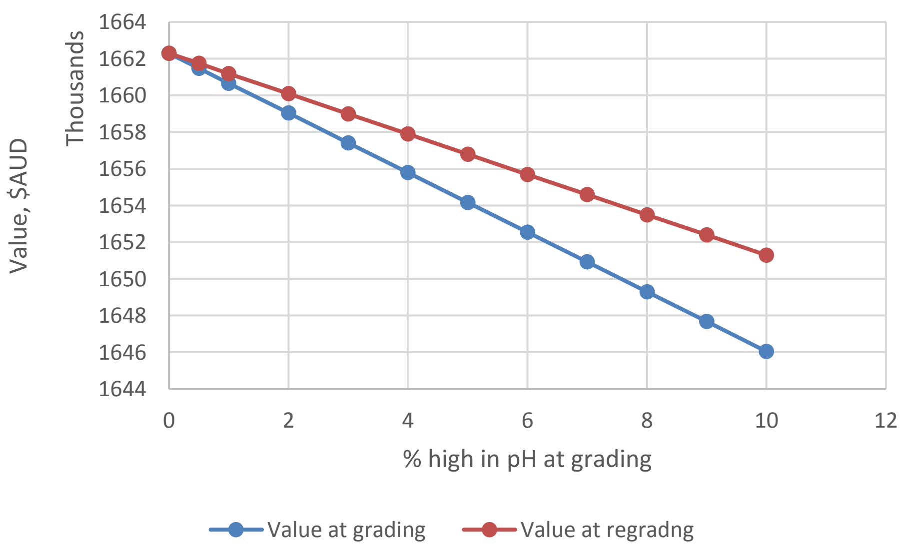

Figure 15. Value of 1,000 beef carcasses at grading, and regrading across a range of high pH % at grading. This calculation uses an incidence of high pH of 8.4% at grading and at re-grading, this incidence drops to 5.7%. This is calculated for 1,000 beef carcasses from animals that were assumed to be 100 days grain-fed and uses an average hot carcass weight of 280 kg.

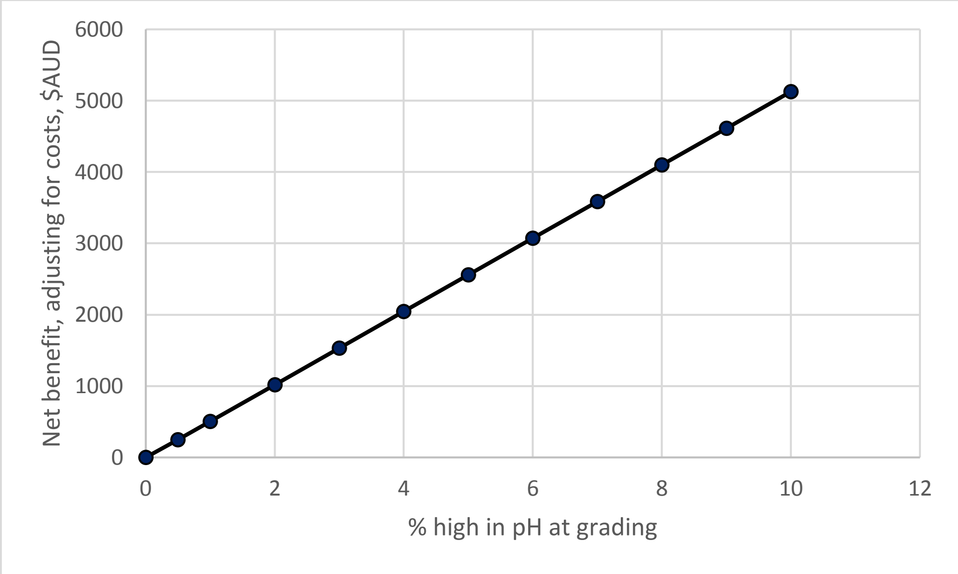

Figure 16. Net benefit of regrading, including costs of labour for regrading. This calculation has the same assumptions and values as figure 1, as well as assuming the time involved in regrading is a set 10 min per day, plus 1 min per carcass, and labour is costed at $65/hr.

5.4 Conclusions

If the incidence of high pH non-compliance when grading at about 12 hrs post-mortem, e.g. 0.5%, there is less benefit in holding carcasses for regrading. But as these data are limited to one processing plant, it would be advised to conduct further data collection across more plants. As the incidence of high pH at grading increases, the value of holding carcasses for a further 12 hrs for regrading also increases. For example, for 1,000 beef carcasses, the net benefit is $5,129 if the high pH incidence is 10% at initial grading, which includes the cost of labour. However even at 2% initial high pH which is standard for most grain fed lots, processors stand to make an extra $1000 per day by re-grading. If the grading occurs later than 12 hrs, the benefit would decrease, as the % carcasses reaching pH compliance at regrading would be lower. Conversely, if the grading occurs before 12 hrs, the benefit would be higher.

6 Discussion

The 2017 Australian Beef Eating Quality Insights reported pH as the main reason for non-compliance for grain-fed cattle between 2015-2017 with incidences between 2.0 and 2.5 % (MLA 2017). Incidences of high pH carcasses observed in the three processing plants included in this study agreed with those values, being similar among plants (2.8 %) and all associated with dark colour meat. However, differences were observed between feedlots being the highest for feedlot L processed at plant B (5.3 %) and the lowest for feedlots K and H (1.2 % each) processed at plant A and B, respectively.

Seasonality and pre- and post-slaughter factors including animal nutrition and temperament, duration of transport, and abattoir management are normally associated with higher high pH percentages (Knee *et al.* 2004; McGilchrist *et al.* 2014). Dark Colour-High pH meat is associated to insufficient glycogen reserves pre-slaughter that leads to inadequate muscle acidification post-mortem and reduced capacity of myoglobin to oxidise and myofibrils to denature producing darker meat that fails to bloom when exposed to oxygen (McGilchrist *et al.* 2014; Ponnampalam *et al.* 2017). Therefore, further analysis of factors determining differences between feedlots (such as transport distance and duration of transport) must be conducted.

Time from slaughter to grading analysis demonstrated that grading at plant A was earlier than in other beef processing plants (< 24 h) for a large proportion of carcasses, and this was associated with higher percentages of **Dark Colour-Normal pH** meat. Results obtained from this study agreed with previous reports that demonstrated that the incidence of dark-coloured beef (with normal pH) can be up to three-fold higher if grading is done 15-18 h post-slaughter compared to grading done 23-26 h post-slaughter (Murray 1989) in agreement with our predictions. Similarly, lighter (higher L*), yellowish and redder (higher a* and b*) carcasses were observed in carcasses graded at 48- vs 24 h post-slaughter (Kirchofer *et al.* 2002). Hughes *et al.* (2014) reported a higher incidence of dark colour meat (8% AUS-MEAT colour score > 3) when carcasses were graded 14 h post-slaughter compared with carcasses graded 31 h post-slaughter (3% AUS-MEAT colour score > 3) (see Table 17). This data again agrees with our prediction. Therefore, darker meat associated with early grading is not new in the literature. Causes for this phenomenon are associated to the incomplete development of muscle colour at earlier times due to the reduced amount of red oxymyoglobin, increased deoxy- and met-myoglobin, decreased lightness, redness and yellowness, and post-mortem metabolic differences among carcasses other than those associated with high pH (Mahmood *et al.* 2017; Hughes *et al.*, 2014, 2017, 2018, 2019a, 2019b; Purlsow *et al.*, 2019).

Table 17. Predicted % of meat colour scores of the beef loin for 1512 beef carcasses graded between 14 and 31 h post-slaughter. Reproduced from Hughes *et al.,* (2014).

Time from slaughter to grading (h)	Meat colour score				
	1B	1C	2	3	>3
14	2	20	40	31	8
19	3	24	41	26	6
24	4	29	41	22	5
26	4	31	40	20	4
31	6	37	38	16	3

Similar to the current study, Mahmood *et al.* (2017) demonstrated that the incidence of **Dark Colour-Normal pH** carcasses was independent of sex, and interestingly, those carcasses were tougher than normal pH carcasses. This indicates that further studies must be conducted on this type of carcasses because, despite being MSA graded, their quality could be inferior to normal colour and normal pH carcasses. It was suggested by Holdstock *et al.* (2014) that steers that produced **Dark Colour-Normal pH** meat had inadequate lairage time to recover from transport. Furthermore, factors that accelerate muscle chilling process such as thinner fat cover may further delay the colour formation and pH declines. Carcass factors, such as reduced fat cover (P8 Fat), low HSCW and low marbling scores were associated with a higher frequency of **Dark Colour-Normal pH** meat in plant A in agreement with previous studies (Murray 1989; Kirchofer *et al.* 2002). In our predictions, carcasses weighing less than 300 kg had twice incidence than carcasses weighing > 300 kg. Similarly, as fat cover increased incidences of dark colour meat were reduced. Therefore, those carcasses can be benefited from delayed grading.

In this analysis, despite the aforementioned effects of HSCW, P8 fat and marbling score on **Dark Colour-Normal pH** incidences, there was no effect of days on feed on the percentages of **Dark Colour-**

High pH meat in disagreement with previous reports that associated high pH with reduced energy storage and reduced glycogen reserves (McGilchrist *et al.* 2012). However, it needs to be acknowledged that for the grading and regrading analysis of cattle from feedlot J processed in plant A, only carcasses from 70 and 100 days on feed were selected and therefore there was a limited range of days on feed. Similarly, the lack of effect of carcass characteristics observed on the percentage of **Dark Colour-High pH** meat in the other processing plants may be due to the low frequency of those characteristics observed in the grading analysis.

The time required to attain ultimate pH is inversely related to muscle temperature and therefore, differences in loin temperature at grading observed among plants in this study may further explain differences in meat pH and colour. It was previously suggested that traditional chilling methods make pH unlikely to be attained between 15 and 24 h, being more likely to be obtained at 48 h post slaughter and that spray chilling may cause and additional delay in pH decline (Murray 1989). Interestingly, spray chilling was implemented in plant A from April 2018. Further analysis of the effect of spray chilling on meat pH and colour must be conducted.

The incidence of **Dark Colour-Normal pH** and **Dark Colour-High pH** meat had an inverse relationship with Temp@pH6, or the temperature of the muscle during the development of rigor mortis, in agreement with the observations made by Hughes *et al.* (2014). Muscles going to rigor at lower temperatures and affected by cold shortening have a higher incidence of dark colour and high pH which is associated with a thinner red oxymyoglobin layer (Renerre 1990). An optimal Temp@pH6 of 25 ˚C has been suggested to reduce the incidences of dark colour and high pH meat (Hughes *et al.* 2014). This is coincidently the temperature that predicted the lowest incidence of **Dark Colour-High pH** carcasses in our study. However, at this temperature, our model still predicted 11 % of **Dark Colour-Normal pH** meat at grading, indicating that factors other than temperature-pH decline may determine this characteristic.

The grading and regrading analysis showed that the average pH of AUS-MEAT colour score 4 at grading was 5.60 explaining the high proportion of carcasses showing **Dark Colour-Normal pH** at grading (21 % for the grading and regrading analysis and 25.5 % for the complete data set). This data indicates that most dark coloured meat carcasses observed at plant A at grading may have represented normal meat with had not yet reached their final colour.

These findings indicate that, despite following the AUS-MEAT guidelines of grading at >8 – 18 h post-slaughter for plants A and C, where electrical stimulation was in place, , when carcasses are graded during the first 24 h post-slaughter and although meat pH was within the accepted range, meat colour may have not reached its ultimate value. The focus should be on defining 'effective' electrical simulation. For example, although plant A was using electrical stimulation during bleeding, it was applied using a very low current (3 mA) and 4.2% of the beef carcasses were defined as cold-shortened (temperature at pH = 6 of <12°C). Hence it is self-evident that plant A did not have a system of 'effective' electrical stimulation.
This condition can be, nevertheless, affected by factors other than those related to pre-slaughter stress and high pH meat (Murray 1989). Previous studies suggested delaying colour measurement beyond 26 h post-slaughter may result in improvements in meat colour (Murray 1989; Hughes *et al.* 2014) in agreement with our study where carcasses from plant B, had lower incidences of **Dark Colour-Normal pH** mainly meat because the majority of grading was conducted >24 h post-slaughter.

AUS-MEAT colour score analysis was confounded by the utilization of different graders for grading and regrading indicating that, although graders are MSA certified, they potentially differ in colour perception. These differences were demonstrated in the analysis of the objective colour values (L*, a*, b*, Chroma and Hue˚) using the NIX Colour Sensor. This analysis showed that, compared to grading, higher objective colour values, indicative of lighter and redder carcasses were obtained at regrading in contrast to the darker AUS-MEAT colour score reported by the grader. The mechanisms responsible to the lightening of meat over time are not fully known, however they can be associated to the progression of pH decline and its effect on myoglobin oxidative status promoting red oxymyoglobin formation and shrinkage of myofibrils increasing light scattering (MacDougall 1982; Hughes *et al.* 2014).

A positive relationship between visual and objective colour determination was previously reported using the Minolta (Klont *et al.* 1999). In the current study we utilized a Nix colour sensor due to its easy use its suitability for meat colour assessment comparable to Minolta and Hunter systems (Hodgen 2016; Holman *et al.* 2018). In the current analysis, dark colour meat (AUS-MEAT score >3) was associated with lower L*, a*, b*, C* and H˚ values than beef with lighter scores in agreement with previous studies using Minolta and Hunterlab (Murray 1989; Hughes *et al.* 2014; Holman *et al.* 2019). Lack of association between objective colour scores and AUS-MEAT colour scores in plant B and C, may be associated with the lower frequency of dark colour carcasses observed in those plants. We suggest, therefore that the industry must use objective colour measurement to confirm the real variation in colour between grading and re-grading based on colourimeter data (NIX, Minolta or Hunter lab). Differences in objective colour values between **Dark Colour-Normal pH** and **Dark Colour-High pH** observed in our study are in agreement with (Mahmood *et al.* 2017) that reported darker meat associated to high pH than to normal pH and attributed those changes to differences in glycolytic potential and muscle post-mortem metabolism.

Nevertheless, the grading and regrading analysis conducted at plant A demonstrated that regrading indeed helps to reduce muscle pH and improving meat colour indicating that by the time of grading (12.6 h post-slaughter) muscle ultimate pH and colour may have not been reached. The regrading analysis demonstrated that 32.3 % of carcasses that were **Dark Colour-High pH** became compliant in pH after regrading, confirming that regrading or delaying the grading in plant A may reduce the incidences of high pH.

The industry needs to recognise that the final meat colour is often not established at <8-24 h post-mortem and carcasses graded as non-compliant for meat colour, may be compliant for meat colour at 48 h post-slaughter. The establishment of acceptable meat colour, in beef loins with normal pH, is a time dependent process, relying on complex biochemical and structural changes (Hughes et al., 2014, 2017, 2018, 2019a, 2019b; Purslow et al., 2019). Further research will help to elucidate these mechanisms and should assist in developing practical strategies for the industry to improve compliance with company specifications around colour, and also industry specifications around pH.

7 Conclusions/recommendations

7.1 Conclusion

Time of grading analysis conducted to 139,703 grain-fed carcasses from seven feedlots and processed in three plants between September 2017 and August 2018 demonstrated similar incidences of Dark Colour-High pH meat among plants (2.8 %) and that earlier time of grading (< 24 h) was associated with higher percentages of Dark Colour-Normal pH meat. Then, grading analysis of 1,181 carcasses demonstrated that the incidence of Dark Colour-Normal pH and Dark Colour-High pH meat had an inverse relationship with Temp@pH6, and that reduced P8Fat, marbling score, HSCW, and time of grading all were associated with high percentages of Dark Colour-Normal pH meat. Finally, when regrading Dark-Colour-High pH carcases, 81.8 % of those carcasses showed a reduction in pH and 7.07 % in colour. Our results suggest that when carcasses are graded during the first 24 h post-slaughter, meat colour and pH may have not reached their ultimate value and that regrading or delaying the grading helps to reduce muscle ultimate pH and to improve meat colour reducing the incidences of high pH.

7.2 Recommendations

We recommend the following;

- Where possible, standardize grading time to more than 20 h post-slaughter to reduce the incidences of Dark Colour-Normal pH and Dark Colour- High pH meat.
- Where grading occurs <20 h post-slaughter, any carcasses graded as high pH must be re-graded.
- Delay the time of grading or regrading for those carcasses showing reduced P8Fat, marbling score, or HSCW, because they may be associated with of Dark Colour-Normal pH meat, or ensure 'truly effective' electrical stimulation is in place.
- Avoid low Temp@pH6 or cold shortening because this may be associated with Dark Colour-Normal pH and Dark Colour- High pH meat, ie. Ensure carcasses are in the MSA pH-temperature window.
- The industry should consider using objective colour measurement to confirm the real variation in colour between grading and re-grading based on colourimeter data, using objective measurements and a chromameter (NIX, Minolta or Hunter lab).
- Further consideration should be given to handling of meat colour chip standards and training of AUS-MEAT/MSA graders, to ensure standardisation between graders.
- The improvement in colour in many beef loins over the period 8-48 h post-slaughter needs to be recognised and the mechanisms investigated, to allow increased compliance to company specifications for colour.
- Further studies are required to determine the metabolic and meat quality differences between Dark Colour-Normal pH and Dark Colour-High pH meat because, despite being MSA graded, their quality could be inferior to normal colour and normal pH carcasses.
- Further studies are required to determine the effects of spray chilling on meat pH and colour and on the incidences of Dark Colour-Normal pH and Dark Colour-High pH meat.
- The economic cost of high pH beef carcasses, and the benefit of regrading, should be communicated to the meat industry, via an industry fact sheet.

8 Key messages

- Processors should standardize grading time to more than 20 h post-slaughter to reduce the incidences of Dark Colour-Normal pH and Dark Colour- High pH meat.
- Where delaying grading to >20 h post-slaughter is not possible, any carcasses with high pH should be regraded at >20 h.
- Processors should delay the time of grading or should implement regrading oforthose carcasses showing reduced P8 Fat, marbling score, and HSCW, because they may be associated with of Dark Colour-Normal pH meat.
- Processors should control the chilling process to avoid low Temp@pH6 or cold shortening because they may be associated with Dark Colour-Normal pH and Dark Colour- High pH meat.
- Beef loins that are non-compliant for colour, but compliant for pH (Dark colour-Normal pH) at early times (<18 h) of grading are very likely to become compliant for colour if graded at 48 h post-slaughter.
- If adopting these measures to regrade beef carcasses, and the incidence of high pH at early grading (12 h post-slaughter) is 10%, the net economic benefits of regrading are $5,129, over 1,000 carcasses.

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