Final report

Lamb meat yield benefit of Rinse & Chill®

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Prepared by: Brad Wilesmith, MPSC Australia; Drs Stephanie Fowler, NSW Dept Primary Industries & Agriculture; and David Rutley, AFMG Pty Ltd & JS Davies Research Centre, The University of Adelaide

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Abstract

Rinse & Chill® (RCT) is a proprietary technology developed by MPSC Inc with the purpose of removing residual blood from the circulatory system of a humanely stunned animal. RCT uses a chilled isotonic solution of dilute substrates. The objective of RCT is to modulate muscle pH and temperature, increasing eating quality and food safety, whilst improving red meat yield. However, to date the impact of RCT on lamb yield has not yet been independently validated.

Consequently, this trial aimed to verify the benefit of Rinse & Chill® Technology (RCT) on the yield and assess whether use of RCT had any impact on the pH decline of lamb carcases. To this end, 1,324 lamb carcases were processed on 5 days over two weeks and traits including pre rinse weight, hot standard and cold carcase weight (HSCW and CCW), GR tissue depth, pH and temperature were measured. Square cut shoulders were boned out from a subset of carcases to assess if RCT treatment had any impact on purge of the forequarter cuts.

Treatment with RCT was found to significantly increase HSCW by 700g/carcase (s.e. ± 48g), which was the equivalent of a 3.2% increase in yield. Likewise, CCW was increased by 700g/carcase treated (s.e. ± 89g). No significant effect of treatment was found on shrink or purge. The pH temperature decline data completed on a subset of carcases demonstrated that treated carcases had a more rapid pH and temperature decline and while they tended to have a lower pH than control carcases, it was no longer significant after the third reading.

Further data is required to investigate the more rapid pH decline on meat and eating quality. Anecdotal observation suggests that the eating quality of RCT lamb is excellent irrespective of the pH decline deviation from MSA requirements. However, there is no hard data to support this observation.

Executive summary

Background

Rinse & Chill® Technology (RCT) has recently been able to be implemented at speeds high enough to be installed into lamb processing chains. As RCT is new to lamb processing its effect on hot standard carcase weight (HSCW) needs to be independently verified and scrutinised. Consequently, a trial has been conducted in order to provide the information required to educate producers, processors and distributors/wholesalers of the HSCW yield benefit of RCT. Note: carcases that have been subjected to the Rinse & Chill® process must comply to AUS-MEAT requirements when traded over the hooks and that no adjustment is to be made to the HSCW for feedback purposes.

Objectives

The aim of this project was primarily to estimate the effect of RCT on lamb HSCW, whilst investigating the impact of the RCT on chiller shrink, pH decline and purge of a forequarter cut, the square cut shoulder.

Methodology

Lamb carcases (n = 1,324) were processed in a commercial abattoir. Of these, 697 carcases were infused approximately 20 min post slaughter while 702 carcases were not (control), in 6 days over a 2-week period (3 consecutive measurement days each week). They were weighed on the slaughter chain prior to rinsing (pre-rinsed weight) and at the weigh grade scale (HSCW).

Immediately post slaughter, pH temperature declines were recorded in the loin. Furthermore, at 24h post mortem, cold weights (CW) were recorded to assess the effect on chiller shrink and square cut shoulders were boned out and aged for two weeks to test for the effect of RCT on purge.

Results/key findings

Lamb HSCW was increased by 700g (s.e 48), similarly CCW was also increased by 700g (s.e ± 89) increasing yield by 3.2% for 22.0 kg carcases. There was no significant effect of treatment on chiller shrink, or purge. The pH temperature decline data completed on a subset of carcases demonstrated that treated carcases had a more rapid pH and temperature decline and pH at 24h tended to have a lower pH then control carcases.

Benefits to industry

This report addresses the effect of RCT on the yield of lamb HSCW and chiller shrink. The secondary aim was to increase our understanding about RCTs effect on purge of the shoulder and the pH temperature decline.

Future research and recommendations

Overall, this trial demonstrates a yield advantage with the use of RCT and indicates a more rapid pH decline is achievable. However, the impact of the more rapid pH decline and tendency for lower pH values on meat quality traits is yet to be investigated. Given previous research has indicated advantages to meat quality are achievable even when pH decline is not optimal, further research on the impact of RCT treatment on pH decline and meat quality is warranted.

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1. Background

1.1 Rinse & Chill® Technology

The additional red meat yield delivered with Rinse & Chill® Technology (RCT) in beef is widely understood and the industry has come to accept that it gives an increased yield in the range of 5 – 6%. This has been established over many years with extensive private investigations as well as a funded study by the Meat Research Corporation in 1997, and subsequently published in MLA papers as recently at 2016 (Polkinghorne 2016). While RCT technology has been adopted in lamb and small stock, there have been no studies undertaken to determine whether this increase in yield is consistent between species.

Opportunities exist to expand the use of RCT within the Australian lamb processing industry as recently a high-speed application machine (HSAM) operating at >13 carcases per minute have been developed. While the company has completed significant yield testing on small stock in collaboration with its early client establishments, nothing has been published to date. Each of these yield evaluations has involved weighing several thousand live lambs, indicating on average an increase in HSCW of between 3 – 3.5% can be expected. However, results need to be independently scrutinised and published before the yield benefits that arise from adoption of RCT for lamb carcases can be recognised.

2. Objectives

The primary deliverable will be a substantive independent and scientifically robust analysis and report that provides reliable information of the impact of RCT on yield outcomes in trade lambs. Secondary to this, an investigation was conducted to assess the impact of RCT on the pH decline and purge of the square cut shoulder.

3. Methodology

3.1 Trial Design

This trial was designed to estimate the benefit of Rinse & Chill® technology for preventing hot standard carcase weight (HSCW) loss in lamb carcases as they are processed on the slaughter floor.

This trial:

- Had a 95% chance of detecting an advantage as small as 200 grams per carcase.
- Collected data on 1,450 carcases, treating 726 with 724 untreated control carcases.
- The experimental design included treating 100 lamb carcases and compared them to 100 untreated control carcases, repeated six times (6 replicates). The exact number by replicates being flexible.
- The experimental design required a pre-rinse weight matched to the HSCW.
- Had a 95% chance of detecting a difference of 1% Purge between RC and Control carcases.
- The experimental design included use of >66 primals, >33 treat and >33 untreated control primals subsampled from RC treated and control carcases, >11 of each per day on three days.

Treatment numbers were determined using G*Power tests with the assumptions that head, hooves and skin is of the order of 15% of the live weight, the residual standard deviation weight was 1.2kg, while the purge was estimated to be 3.5% (± 1.2%) for treated and 4.5% (±1.9%) for control.

The G*Power software package version 3.1.9.7 (Faul *et al.*, 2007) was used to describe the relationship between significance and number of observations required.

Depending on the approach of the analysts it was expected that either a General Linear Modelling or General Linear Mixed Modelling routine would be used to analyse the data and estimate the effect of the RCT treatment compared to the untreated control.

3.1.1 Historical Data and Detectable Difference

The Sheep CRC Information Nucleus Flock (INF) (van der Werf *et al.*, 2010) data was used to estimate the residual variance and standard deviation of HSCW adjusted for pre-slaughter live weight. It is assumed that this pre-slaughter live weight would be very highly correlated with a pre-rinse slaughter weight, noting that the head, hooves and skin will have been removed from the body for the pre-rinse slaughter weight. Anecdotal experience suggests that the head, hooves and skin is of the order of 15% of the live weight. It is expected that this weight loss over the early half of the slaughter chain will be variable between lots but consistent within lots.

The INF data from 2007-10 consisting of 8,601 lambs had a HSCW residual standard deviation of 2.46 kg when adjusted for Flock, Year, Kill Group and Sire and Dam Breed. When HSCW was also adjusted for Live Weight this residual standard deviation was reduced to 1.13 kg. To be conservative for the trial design the residual standard deviation used for the calculations in this report was 1.2 kg.

Based on analysis of the historical data, it has been estimated that the limit of detection is 200g per carcase.

3.2 Data collection

3.2.1 Carcases Slaughtered and Sampled

Lambs were slaughtered and dressed and their carcases were measured at a Victorian lamb processing plant over 8 days, with between 248 to 300 carcases processed for the trial each day. Carcases were collected from commercially available lots and processed as per normal standard plant procedures. There was no electrical carcase stimulation, spray chilling or any other processing intervention.

3.2.2 Treatments

Carcases were assigned to the RCT or control groups via random block allocation, where half the carcases assigned to the trial were allocated to either the control or treatment. Whether the treatment or control was conducted first or second was alternated daily to mitigate the risk of a kill order effect.

The solution volume infused was based on liveweight calculated for every individual carcase using liveweight determined from the pre-rinse weight (pre-evisceration weight), as per normal RCT operating procedures.

Carcases which were damaged during processing were noted and excluded from the trial.

3.2.3 Carcase measurements

Carcase measurements taken during the trial included pre-rinse weight, HSCW, pH decline, GR tissue depth, CCW and purge of a square cut shoulder.

Pre-rinse weight was completed before the carcases entered the HSAM footprint. The front hocks were lowered out of the forequarter hangers (Figure 1), accelerated forward of the pusher and onto an inline track scale. The carcases were weighed at this point electronically using an iPad connected to the HSAM PLC when a capture weight button was pushed. A second person writing down the weights for backup and correlation cross examination (Figure 2). When the pusher had caught up and pushed the carcase off the scale the forequarter hocks were rehung into the forequarter hanger. This methodology gave a true hind leg hang only weight the same as the methodology used at the HSCW weigh grade scale. This weight was recorded after the head, hooves and skin were removed to remove any variation caused by these, thus increasing the accuracy of the weight with respect to the true weight of the carcase. Long carcases which touched the floor resulting in an inaccurate weight were noted and excluded from the trial.

Figure 1. Carcases being taken out of forequarter hanger for weighing.

Figure 2. Carcase being weighed, pre-rinse.

HSCW was recorded on entry to the chillers after trimming as per standard industry process (Figure 3). During the study there were 126 carcases that entered the retain rail. Due to possible trimming from pathology/contamination etc these carcases were removed from the carcase yield estimations.

Once in the chiller, the GR tissue depth was measured using a standard GR knife along the 12th rib 110mm from the midline.

Figure 3. Recording of HSCW at chiller entry.

Of the carcases weighed, pH decline was measured in 198 carcases (~50 per day, representing 25 treated and 25 control carcases, over 4 kill days). To this end, the left *M. longissimus thoracis* was measured at the 12th/13th rib site using a pH metre with temperature compensation (WP-80, TPS Pty Ltd., Brisbane, Australia) and a polypropylene spear-type gel electrode (Ionode IJ 44) calibrated at ambient temperature using two pH buffers (pH 4.01 and pH 6.86). Carcases were measured over 6 intervals until carcase temperature dropped below 18°C resulting in up to 7 measures pre-rigor (pH 1 – 6) and subsequent measures were also taken at 24h (pH24).

At 24 h post-mortem, carcases were reweighed for CCW and a subset of these carcases had further samples collected to determine the impact of the RCT on purge. To this end, carcases were weighed at 24h post slaughter before the shoulder (square cut shoulder) was removed and weighed. Once weighed, the square cut shoulder was vacuum packaged and aged for 14 days as per standard practice with 4 per carton in the plant under commercial chiller conditions. After ageing, shoulders were removed from the vacuum pack, dried with paper towel and reweighed to determine purge loss. Purge loss was calculated as the loss from the pre-purge weight calculated as a percentage, below.

The data collection issues identified during the study are given in Appendix 9.1.

3.3 Statistical Analysis

3.3.1 Exploratory modelling

Initial calculation of summary statistics confirmed the data integrity and allowed removal of observations such as retained carcases where unknown trimming may reduce yield and influence the effect of RCT. To ensure accuracy of the results reported and the results were not affected by conflicts of interest, the initial modelling was undertaken independently by two different analysts. Exploratory analysis was completed using both R Statistical Software (R Core Team, 2021) and SAS (SAS Institute Inc, 2017).

3.3.2 Final Analysis

To assess the impact of RCT on HSCW and yield, carcases which had been retained on the pathology rail were removed from the data prior to modelling. Prior to statistical analysis, chiller shrink and purge were calculated based on the equations below.

Chiller Shrink was calculated as 100% less the ratio of CCW/HCW

$$Chiller\ Shrink = 100 - \left(\frac{CCW}{HSCW} * 100\right)$$

Purge, in the vacuum pack, was calculated using the formula below

$$Purge = 100 - \left(\frac{Post\ Purge\ Wt}{Pre\ Purge\ Wt} * 100\right)$$

Linear models and ANOVA were used to calculate predicted means for HSCW, CCW and shrink using kill as a fixed effect and pre- rinsed weight as a covariate. Therefore, the effect of treatment could be predicted at an average pre-rinsed weight given an unintentional difference in mean pre-rinsed weights were noted between treated and non-treated carcases. Given the effect of treatment on HCW, further models to assess the impact of treatment on the CCW and shrink did not include HSCW to avoid removing the known treatment effect.

As pre-rinsed weights, HSCW, CCW and pre-purge weights are highly correlated, heavier shoulders (pre-purge weights) are obtained from heavier carcases, purge was modelled using only kill as a fixed effect. This also accounted for the shoulders from one kill which spent one more day (15) in the chiller while all others spent 14 days in the chiller before the post purge weight was recorded and the “batch” effect caused by day-to-day differences in the chiller temperatures.

Linear models, ANOVA and predicted means were calculated for pH measured at each time point to estimate the treatment effects, while a spline was used to predict the Temp at pH6 and the pH at Temp 18°C to demonstrate the effect of treatment on the overall pH decline. This was completed using the model described by van de Ven *et al.* (2014), where

$$pH_{ij} = spline\left(T_{ij}\right) + a_i + b_i T_i + error_{ij}$$

All analyses were completed in R Core Software, using the *‘lem4*’, *‘asreml’* and ’*lmerTest*’ packages while graphics were completed using *‘ggplot*’ (R Core Team, 2021). Raw statistical output is presented in Appendix 9.2.

4. Results

4.1 Carcase Yield Traits

Summary statistics for the carcase traits by treatment are outlined in Table 1.

Table 1 Summary statistics for carcase traits by treatment and kill date.

Kill Date by Treatment	Pre-Rinsed Weight (kg)			HSCW (kg)			CCW (kg)			GR Tissue Depth (mm)		
	Mean (± s.d)	Range (min – max)	Count	Mean (± s.d)	Range (min – max)	Count	Mean (± s.d)	Range (min – max)	Count	Mean (± s.d)	Range (min – max)	Count
8/12/2021												
Control	36.2 (4.0)	27.1 – 46.0	104	23.5 (2.8)	17.8 - 30.1	104	22.7 (2.6)	18.1 - 29.3	55	10.8 (3.8)	4 - 22	104
RCT	35.8 (3.7)	26.4 - 44.8	116	24.0 (2.8)	17.0 - 31.4	116	23.1 (2.6)	18.5 - 30.7	50	11.7 (4.3)	3 - 25	116
9/12/2021												
Control	33.7 (3.6)	25.4 - 44.8	139	21.3 (2.6)	14.9 - 29.2	139	21.2 (2.6)	15.6 - 28.5	27	8.9 (3.8)	0 - 20	139
RCT	33.8 (3.8)	24.6 - 44.7	137	21.7 (2.9)	14.5 - 28.3	137	21.9 (1.6)	16.3 – 25.0	47	7.4 (3.3)	0 - 19	137
14/12/2021												
Control	35.1 (2.8)	29.1 - 42.2	144	22.7 (2.4)	16.4 - 29.6	144	22.0 (2.1)	18.9 - 27.3	27	10.4 (4.1)	3 - 25	144
RCT	35.2 (2.8)	27.6 - 41.5	136	23.4 (2.2)	17.9 - 29.3	136	22.8 (2.1)	19.2 - 26.1	29	11.2 (3.9)	3 - 25	136
15/12/2021												
Control	33.7 (4.2)	25.5 - 46.5	134	21.8 (2.9)	16.5 - 30.4	134	21.6 (3.1)	16.5 - 28.2	28	9.1 (3.6)	4 - 20	134
RCT	33.4 (4.1)	25.4 - 46.9	136	22.4 (3.1)	15.0 - 32.6	136	21.3 (2.7)	17.2 - 27.7	28	9.7 (3.9)	1 - 24	136
16/12/2021												
Control	32.6 (3.5)	25.9 - 41.4	139	21.2 (2.6)	15.6 - 28.1	139	20.5 (3.0)	15.2 - 27.6	28	8.1 (2.8)	3 - 16	139
RCT	32.1 (3.7)	24.7 - 40.8	139	21.6 (2.8)	15.4 - 28.6	139	21.6 (2.5)	17.4 - 26.7	28	8.9 (3.2)	3 - 23	139
Overall												
Control	34.2 (3.8)	25.4 – 46.5	660	22.0 (2.8)	14.9 – 30.4	660	21.8 (2.8)	15.2 – 29.3	165	9.4 (3.8)	0 – 25	660
RCT	34.0 (3.9)	24.6 – 46.9	664	22.6 (2.9)	14.5 – 32.6	664	22.2 (2.3)	16.3 – 30.7	182	9.7 (4.0)	0 – 25	664

4.1.1 Hot Standard Carcase Weight

After removing carcases that were retained on the pathology rail 1,324 observations were obtained over five days. Pre-rinsed weights did not differ significantly between the RCT and control groups (Table 1). Consequently, adjusting the models to a mean pre-rinsed weight indicated treatment with RCT increased HSCW by 700g (s.e. ± 48), with a predicted mean HSCW of 22.7kg (95% CL 22.56 – 22.74kg) while the mean weight of control carcases was 22.0kg (95% CL 21.88 – 22.07kg) as shown in Figure 4. The model fitted explained 82% of the variation in HSCW, leaving a residual standard deviation of 1.2kg. Although, HSCW were significantly different between kills, there was no interaction between treatment and kill.

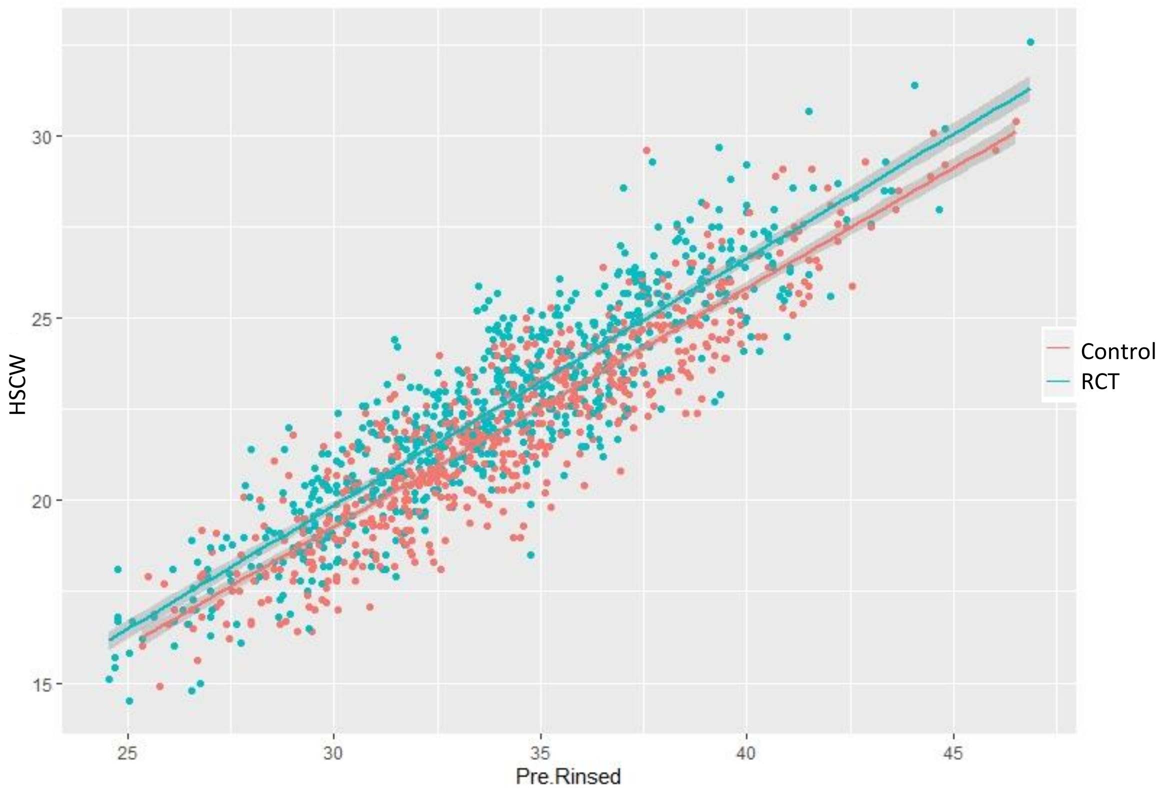

Figure 4. Pre rinsed weight (kg) and HSCW (kg) for RCT and control carcases.

4.1.2 GR Tissue Depth

From the Summary Statistics (Table 1) it can be seen that small differences in GR Tissue Depth were found between treated and control carcases on each date. However, these differences were small (not significant) compared to the variation with treatment group. This was the case across all dates and for individual dates except for the 9/12/2021. Thus, as expected, it can be seen that treatment did not affect GR Tissue Depth.

4.1.3 Cold Carcase Weight

Cold carcase weight was recorded for 346 carcases over the five days. Linear models using pre-rinsed weight as a covariate demonstrated use of the RCT resulted in a mean cold weight of 22.4kgs (95% CL 21.5 – 21.8) compared to a mean CCW of 21.7kg (95% CL 22.2 – 22.5), equating to an increase of 700g/carcase treated (±s. e 0.89) as illustrated in Figure 5. The model fitted explained 82% of the variation in HSCW, leaving a residual standard deviation of 1.1kg. Although, CCW were significantly different between kills, there was no interaction between treatment and kill.

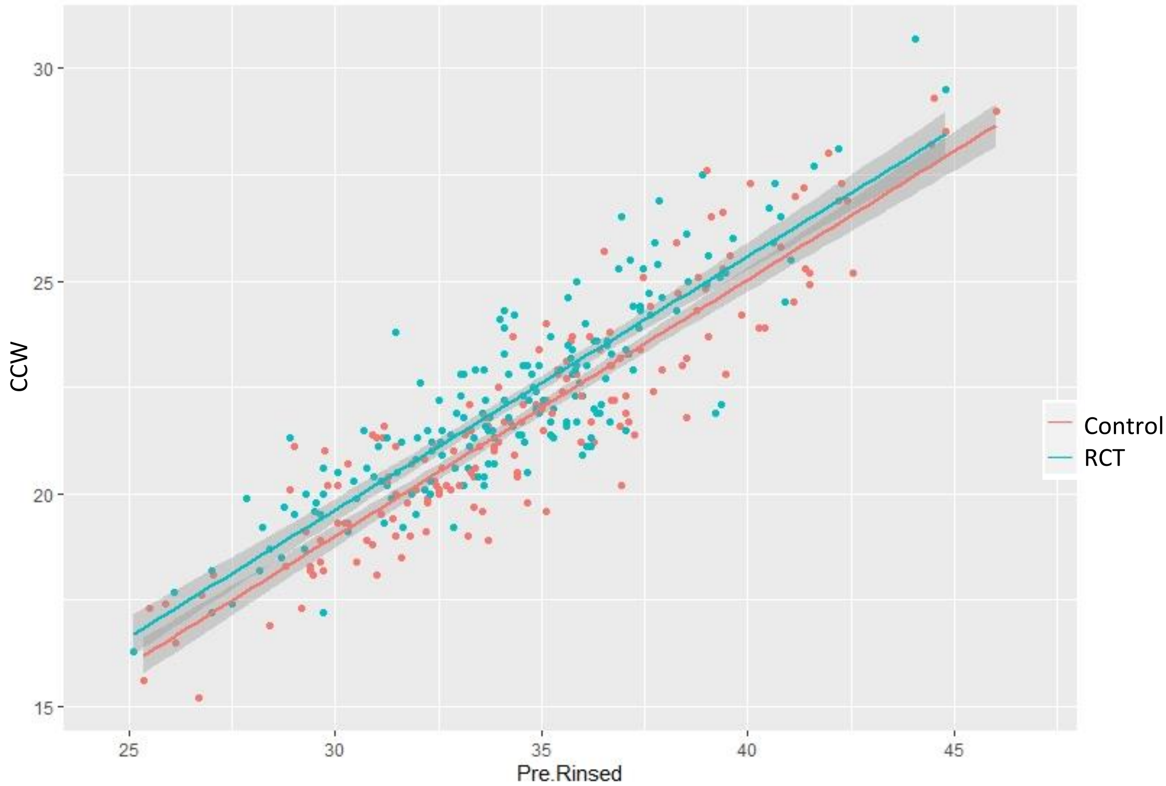

Figure 5. Pre rinsed weight (kg) and CCW (kg) for RCT and control carcases.

4.1.4 Chiller Shrink

Chiller Shrink was recorded for 346 carcases, of which none had been retained on the pathology rail. While chiller shrink was not significant at the *P>0.05*, it was significant at *P >0.1* level (*P* = 0.062). Overall, RCT treated carcases had a mean chiller shrink of 2.61% (95% CL = 2.52 – 2.70, ± s. e. 0.04) while control carcases had a mean chiller shrink of 2.49% (95% CL = 2.40 – 2.58, ± s. e. 0.04). The fitted model explained 15.5% of the variation in shrink, leaving a residual standard deviation of 0.56%.

Chiller shrink is very important to processors. As such it would be prudent to undertake further research on the effect of RCT on Chiller Shrink. Further research would determine if this difference is a function of random sampling or indicative of a systematic effect of RCT.

4.2. Purge Loss

Purge loss was recorded for 72 lamb shoulders vacuum packed and stored in the abattoir's chiller (0 to 0.5 ^{o}C) for two weeks (14 or 15 days), summary statistics are given in Table 2. Modelling demonstrated the mean purge loss for square cut shoulders from RCT treated carcases was 0.86% (95% CL = 0.79 – 0.93), while the mean purge loss for square cut shoulders from control carcases was 0.89% (95% CL = 0.82 – 0.96; ± s.e. 0.03). This model represents 10.9% of the variation in purge, leaving a residual standard deviation of 0.21%. The summary statistics for purge loss can be seen by date in Table 2. The small differences are a function of random sampling as none of the differences were close to significant.

It should be noted that purge loss in this trial is likely to be low. This is due to the study of the square cut shoulder, a large primal with subcutaneous fat on one side and the membrane covering the ribs on the other, while at the same time having small cut surfaces from where purge could be lost. Other effects such as cold shortening may also influence purge loss.

Table 2. Summary statistics for purge loss by treatment and kill.

Kill	Treatment	Purge (%) Mean (± s.d)	Range (min – max)	Count	Days Ageing
8/12/2021	Control	0.88 (0.18)	0.67 – 1.28	12	14
	RCT	0.97 (0.22)	0.64 – 1.33	12	
9/12/2021	Control	0.89 (0.18)	0.62 – 1.18	12	14
	RCT	0.88 (0.25)	0.17 – 1.08	12	
15/12/2021	Control	0.91 (0.20)	0.67 – 1.28	12	15
	RCT	0.74 (0.22)	0.41 – 0.99	12	

4.3 pH/Temperature decline

4.3.1 pH measures

Predicted means for pH measured at each time point as well as Temp at pH6 and pH at Temp 18 as determined by the spline are given in Table 3., while the raw pH data is illustrated by Fig 6.

Table 3. Predicted means (± s.e) for pH measures by treatment.

Treatment	pH #1	s.e.	pH #2	s.e.	pH #3	s.e.	pH #4	s.e.	pH #5	s.e.	pH #6	s.e.	pH 24	s.e.	Temp @ pH 6	pH @ Temp 18
RCT	6.75 a	0.04	6.46 a	0.03	6.32 a	0.05	6.23	0.05	6.17	0.05	6.09	0.06	5.70	0.04	13.0	6.12
Control	6.95 b		6.95 b		6.41 b		6.27		6.19		6.13		5.73		14.1	6.15

pH means indicated by the letter a or b differ significantly (P<0.05)

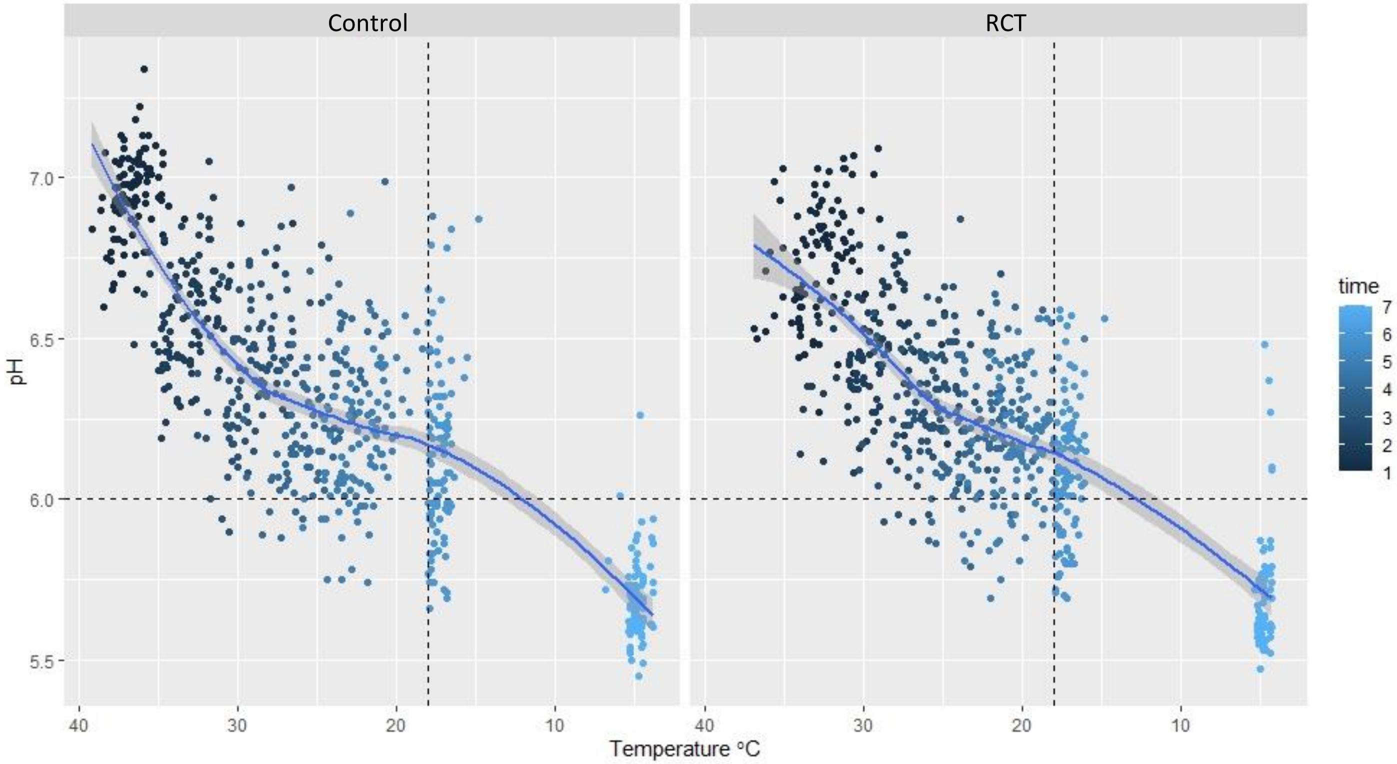

Figure 6. Raw pH and temperature measures for RCT and control carcases.

Predicted means for pH data measured at each time point demonstrates RCT treated carcases had a significantly lower pH for the first 3 measures, while they tended to be lower for the remaining measures, the difference between treated and control carcases was not significant.

4.3.2 pH/temperature decline models

The modelling pH decline using splines predicts the carcases would not have fallen to pH 6 until RCT treated carcases were 14.1°C (95% CI= 13.0 – 15.3°C) while the control carcases would not have fallen to pH 6 until they were 13.1°C (95% CI = 12.1 – 14.1°C), as illustrated in Figure 7. This model predicts carcases would have reached temperatures of 18°C when the pH of both RCT treated and control carcases was at 6.12 Although the 95% CL of RCT carcases was slightly wider with pH values of 6.07 – 6.16 compared to 6.11 – 6.16 for control carcases.

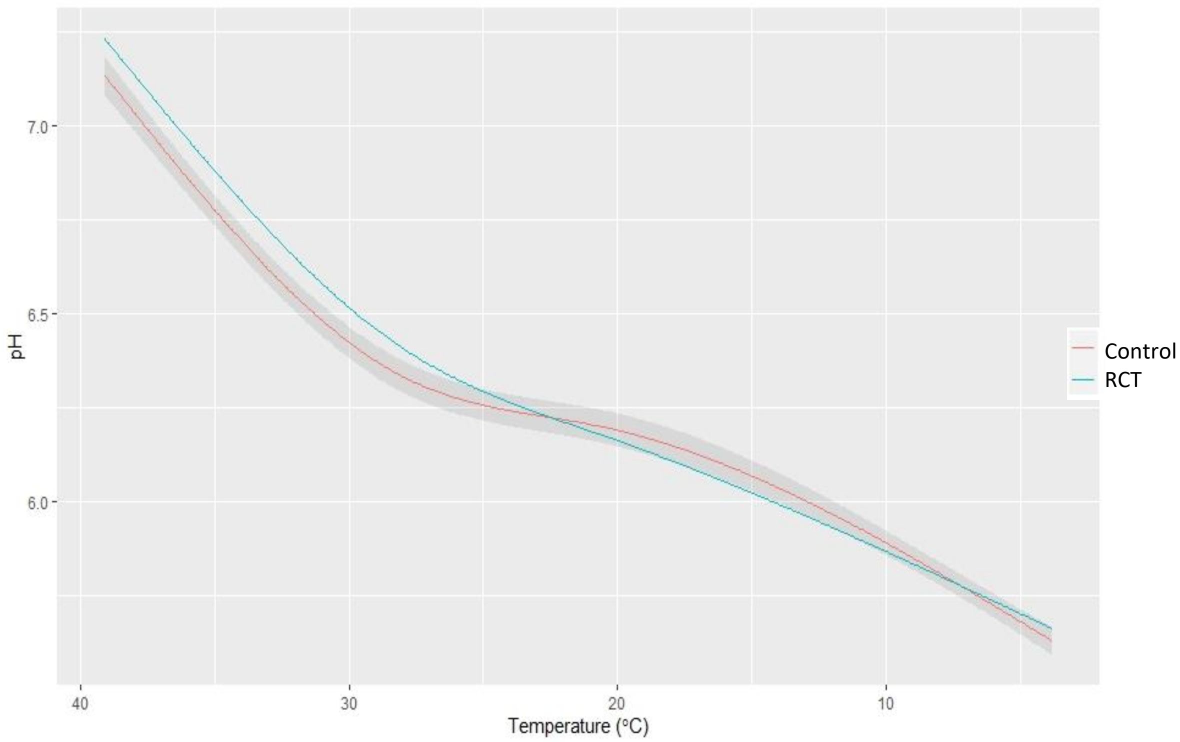

Figure 7. pH/temperature decline as modelled using the spline to predict the temp at pH6 and pH at temp 18°C.

5. Discussion

Overall, this trial demonstrated HSCW was increased by 3.2% when RCT was used. While there is a paucity of research in lamb, this is consistent with research on beef carcases which has shown early post mortem changes to the muscle as a result of RCT causes an increase in carcase yield of between 2 – 4% (Dikeman *et al.*, 2003; Moreira *et al.*, 2018; Yancey *et al.*, 2001). Although this effect is poorly understood, Dikeman *et al.* (2003) have hypothesised that increases in yield are the result of fluid retention which may have been achieved by altering the pH and temperature decline of carcases during the early post mortem period. This is achieved as the substrates in the solution are metabolised by the muscle during the early post mortem period and when chilled, results in more effective blood removal, a better pH decline and more efficient carcase chilling (Hwang *et al.*, 2022).

The relationship between pH decline and water holding capacity has been well described in meat science. Indeed, a review by Hughes *et al.* (2014) highlights the role of pH, temperature and degree of glycolysis in defining the myofilament lattice arrangement, denaturation of the myofilament heads and subsequently the extent of sarcomere shortening. This is critical to the water holding capacity of meat as water is pushed into the extracellular space when protein connections are compromised because the force of the shrinkage during rigor is transferred to the whole muscle. Thus, in order to retain moisture, protein connections are required to remain intact to prevent the shrinkage being translated from the myofibrils to the whole muscle (Hughes *et al.*, 2014). Given the use of RCT decreases initial carcase temperatures, as demonstrated by the pH/temperature decline data and increases early post mortem glycolysis, it is postulated that RCT reduces the deformation of myosin which occurs at a greater rate when pH falls while temperatures are still high (Hughes *et al.*, 2014). Therefore, it is plausible that RCT treated carcases retain more water during the early post mortem period while carcase temperature remains high resulting in a greater HSCW when compared to non-treated carcases. However, further research is required to confirm the mechanism.

Lamb is prone to meat quality issues as a result of chilling, given its susceptibility to excessive evaporative weight loss as a consequence of high surface to volume ratio and thin relatively exposed musculature which can lead to rapid rates of chilling (Brown *et al.*, 1993). Studies assessing the impact of chilling regime on meat quality have demonstrated conventional chilling of carcases can result in weight losses of up to 2.5% (Brown *et al.*, 1993), which is consistent with the current trial where carcases were not subjected to spray chilling. The difference between the RCT and control carcases was not significant, indicating the 3.2% gain in HSCW is not lost during the chilling process. This is confirmed by the analysis of mean CCW, which showed CCW of RCT carcases was also ~700g greater than non-treated carcases. It is important to note, that while HSCW and CCW were significantly different between kills, there was no interaction between kill and treatment indicating that the increase in weights achieved with RCT treatment was independent of the kill and therefore, the benefit would be expected to remain consistent across days and lots regardless of the carcases treated.

Purge of forequarter cuts has also been identified as a potential issue with the RCT system given lamb carcases have a high surface to volume ratio and thinly exposed musculature, making them susceptible to cold shortening (Brown *et al.*, 1993) and the RCT is a vascular rinse system which uses the chilled solution (Hwang *et al.*, 2022). Although it was acknowledged in the project design that samples from larger primals, such as a square cut shoulder were expected to have a lower purge than the cuts which the trial design was based on (loin and topside), purge (0.9% s.e. ± 0.2) was less than the assumed 3.2%. While this agrees with previous research which suggests purge does not vary between RCT and non-treated carcases (Fowler *et al.*, 2017; Dikeman *et al.*, 2003), there are also a number of studies which suggest higher purge can result from treatment (Farouk and Price, 1994; Farouk *et al.*, 1992). However, these studies differ in the composition of the infusion which may explain the variation in results. The infusion used by Dikeman *et al.* (2003) consisted of saccharides, sodium chloride and a phosphate blend which is similar to the current trial while the studies conducted by Farouk *et al.* (1992) and Farouk and Price (1994) infused carcases with a blend of sodium chloride and calcium chloride and sodium chloride alone. Given that Dikeman *et al.* (2003) have demonstrated inclusion of calcium chloride induces greater shortening of the myofibril, it is likely the increased purge found in the two studies completed by Farouk was a result of the blend infused.

While this trial agrees with Fowler *et al.* (2017) who found no difference in purge from the loin with a similar solution, the numbers measured for purge in the current trial are too low to detect a significant difference in purge for a square cut shoulder. While the purge from the square cut shoulder has no practical significance given the small purge loss, it cannot be concluded from this trial whether purge would be increased if shoulders were boned out into primals, given the square cut shoulder has less exposed surface area when compared to alternate shoulder cuts as it has subcutaneous fat on one side and the membrane covering the ribs on the other. As the size and weight of lambs has increased and household sizes are decreasing, large cuts such as the square cut shoulder are becoming less popular with consumers due to their large size and associated cost and consequently value-added lamb cuts are being increasingly used to maintain consumer appeal (Fowler *et al.*, 2018). Therefore, further work is warranted to ensure no difference in purge is experienced for shoulder cuts from treated carcases when there is more surface area of the muscle exposed to the forces of vacuum packaging. A University of Melbourne study (Ha, *et al.*, 2020 confidential report, unpublished) found no difference in purge loss at up to 105 days of post slaughter age between RCT and control loins (*longissimus lumborum et thoracis*). Though not a forequarter cut this muscle extends into the shoulder, having been fabricated from the loin by removal of all bone, fat and muscle membranes, commonly sold as a fully denuded product.

The relationship between tenderness and pH decline has been well defined and collectively it is agreed that antemortem conditions which result in a pH decline which is too rapid or too slow while carcases cool both result in significantly shortened muscles as noted by decreased sarcomere lengths (Smulders *et al.*, 1990). As a result, recommendations to industry have been made by Hopkins *et al.* (2015) with a target of pH6 when carcases are between 18 – 35°C giving industry a pH and temperature "window" to ensure meat quality. Spline modelling from the current study has highlighted that all carcases in the current study would be defined as "cold shortened" as the pH remained high as the carcases cooled noted by the prediction of reaching pH6 when RCT carcases were 14.1°C while the control carcases would not have fallen to pH 6 until they were 13.1°C. Although medium voltage electrical stimulation has been used to increase the early pH decline and therefore increase the proportion of carcases which attain a pH of 6 between 18 - 35°C (Pearce *et al.*, 2009), the carcases in the current study were not subjected to electrical stimulation and consequently further research is required to ensure that the use of RCT combined with electrical stimulation does not induce heat shortening given the pH decline was more rapid during the early post mortem period when carcases were treated.

The predicted mean pH and temperatures in this trial aligns with previous research which indicates the rate of decline is greatest during the early post mortem period i.e. the first 45 min post slaughter, when muscle temperatures are highest (Holman *et al.*, 2021). This is supported by previous research conducted in beef by Dikeman *et al.* (2003) who indicated the pH dropped more rapidly in the first 4 h post mortem for beef and Kethavath *et al.* (2022) who found an increase in the overall rate of pH decline when cull cows were infused with a vascular rinse. While there is a paucity of data in lamb, treatments in the study conducted by Farouk *et al.* (1992) when lamb carcases were infused with sodium chloride yielded a more rapid pH decline when compared to other treatments. Yet findings of Fowler *et al.* (2017) using a similar solution are conflicting, suggesting no improvement to pH decline was found with RCT infusion, although the rate of chilling was suboptimal suggesting further research is required to ascertain the repeatability of the findings in different chilling regimes.

The early post mortem period is critical to meat quality as it is well established that tenderness is affected when glycolysis is slow (Smulders *et al.*, 1990). Although the mechanisms are poorly understood, it is evident from this trial that pH decline is expedited with the use of RCT, which may increase tenderness as it is plausible the substrates in the solution increase the glycolytic rates of the muscle by acting as if it was glycogen as it is metabolised by the muscle. Although Fowler *et al.* (2017) used a similar solution, assessment of the pH declines given in the paper suggests carcases in that trial underwent suboptimal processing and remained at a high temperature for much of the early post mortem period, resulting in heat shortening while the carcases in the present trial dropped temperature rapidly during the early post mortem period which may account for differences between trials. Interestingly, despite the heat shortening of all carcases included in the trial, Fowler *et al.* (2017) found a reduction in shear force of lamb from carcases which had been infused with the RCT, suggesting that improvements to eating quality may be possible with RCT infusion when processing does not meet the optimal pH/temperature "window". Whether RCT is able to overcome the impact of poor processing techniques on eating quality is yet to be confirmed but further research is warranted given the demonstrated increase in yield.

It has been considered that the effect of RCT on rapidly cooling the carcase on the slaughter floor could enhance water holding capacity and so improve eating quality according to the reviews of Cheng and Sun (2008) and Warner (2017). A recent study indicates that RCT does not affect water holding capacity in lamb cuts during storage and cooking (Li *et al.*, 2023), whether water holding capacity is affected in the carcase on the slaughter floor is not known. In their review, Hwang *et al.* (2022) consider the effect of RCT on the rate of pH decline discussing the effect of the RCT solution substrates on anaerobic metabolism, lactic acid build up and pH decline. However, the US review does not consider pH decline with respect to the pH by Temperature decline rate but rather the rate of pH change over time. The current state of the literature suggests that this is an area raising challenging questions, requiring further research.

6. Conclusion

Overall, this study demonstrated treatment of lamb carcases with the Rinse & Chill® resulted in a significant increase of lamb yield, which is retained through chilling. Although carcases did not meet the pH/temperature decline window, treatment with Rinse & Chill® accelerated the decline in pH during the early post-mortem period, suggesting that further research to understand the implications on eating quality is warranted.

6.1 Key findings

1. Treatment with Rinse & Chill® increased lamb HSCW and CCW, resulting in an increase in carcase yield by 3.2% on average.
2. Chiller shrink and purge on a square cut shoulder was not significantly different between treated and control groups suggesting this gain in yield is maintained through further processing. It must be noted that the large size of the square cut shoulder with small cut surfaces compared to the area of subcutaneous fat and muscle membrane suggest that this may not be representative of other lamb cuts. Also, the cold shortening may have reduced any differences.
3. Treatment with Rinse & Chill® accelerated the early post-mortem pH decline suggesting it may overcome the impacts of suboptimal processing, such as heat and cold shortening, on eating quality.

6.2 Benefits to industry

Over The Hooks (OTH) trading is a procurement system that uses the Hot Standard Carcase Weight (HSCW) to calculate payment based on an agreed price ($/kg). The RCT process, classified as a processing aid, takes place prior to the Hot Weight Carcase Scale, therefore any yield benefit from the RCT process it is assumed would transfer to the producer during OTH trading. However, it is the processor that invests in the technology. This is the major reason processors don't rinse 100% of the stock processed and in turn magnifies inconsistencies in product. AUS-MEAT has recommended, when a processor uses RCT, that OTH procurement can still take place via a commercial agreement between the Processor and Producer using a transparent system to identify RCT yield gain.

1. This independently scrutinised trial provides transparent industry understanding of the RCT effect on HSCW and lamb carcase yield, as a basis for assigning value when trading lambs over the hooks (OTH).
2. Transparent and improved understanding will allow producers and processors to establish fair trading terms for trading lambs OTH when the abattoir uses RCT.
3. Appropriate allocation of value will allow processors to adopt RCT for lamb carcases, providing all with the benefits of RCT. Adoption of RCT will allow the whole value chain to prosper from improved yield and potential improvements to meat and eating quality, which remain to be verified.

7. Future research and recommendations

1. OTH trading terms need to be developed that acknowledge the yield advantage provided by RCT. It is necessary that these trading terms acknowledge the contributions of all required to implement HSAM RCT and so reward the risks taken.
2. Education programs and literature targeting producers and processors through to retailers need to be developed and disseminated. These resources will explain the potential to

enhance lamb carcase yield and how this benefit can be equitably disbursed to producers and processors. Upon adoption, economic supply and demand forces will disburse the rewards appropriately through the value chain.

3 As there is no published information and little understanding of the bio-physical and chemical mechanisms underpinning the advantages of RCT, neither the response in the pH temperature decline or eating quality can be predicted. Further independent research is recommended to understand potential impacts on key quality traits.

4 Future R&D should include the publication of unpublished data in peer journals, increasing the body of scientific knowledge on the benefits of RCT to beef and lamb products. Some of this data demonstrates the value of RCT on retail display shelf life and consumer sensory analysis.

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9. Appendices

9.1 Data collection and issues

The overall data collection went very well. However, issues arose with the pre-treatment carcase weighing, cold weight, pH decline and purge data collection. These issues were dealt with as follows:

The data from the 7/12/2021 has been excluded from analysis as an issue with rehanging carcases too early affected the pre-rinse weights and the consequent rinse volumes. Consequently, these carcases were not deemed to have been properly rinsed and erroneous carcase weight and purge differentials were expected compared to the untreated control carcases.

Following this issue daily trial numbers were increased to ensure sufficient data were collected to meet the trial objective, 725 RCT and 725 Control carcases. Due to this issue care was taken to treat and collect sufficient data 1,450 carcases were used (>1,200) excluding 126 retained carcases. Data were collected on 1,324 carcases, 664 treated and 660 untreated controls over the following five days to satisfy the primary aim of the trial.

The study design included cold weights from all 1,200 carcases, this was not achievable. Due to Covid and site staff shortages the site was unable to process enough carcases to have a buffer for us to be able to measure all cold weights. We were only able to collect cold weight data on 365 carcases.

Initial purge data was collected from carcases which were then excluded from the trial, consequently data was measured on another 22 samples although due to a subsequent error these samples had had 1 extra day of chilling.

On the 15th December the pH meter probe failed after recording 11 ultimate pH measurements from the carcases processed the previous day. No further pH measurements at 24 h post mortem could be recorded, nor could the pH decline be recorded for any carcases processed on the 15/12/2021.

9.2 Statistical Output

9.2.1 HSCW

HSCW model fitted using Pre-Rinsed Wt & Kill Date

```
Residuals:
    Min      1Q  Median      3Q     Max 
-4.8268 -0.8043  0.0017  0.7689  5.2989 

Coefficients:
                     Estimate Std. Error t value Pr(>|t|)    
(Intercept)         -0.748329   0.354912  -2.108  0.03518 *  
TRTT                 0.713743   0.164183   4.347 1.49e-05 ***
kill9/12/2021       -0.554622   0.159194  -3.484  0.00051 ***
kill14/12/2021      -0.103352   0.156731  -0.659  0.50974    
kill15/12/2021      -0.037019   0.160447  -0.231  0.81756    
kill16/12/2021       0.147966   0.160974   0.919  0.35816    
Pre.Rinsed           0.669847   0.009245  72.455  < 2e-16 ***
TRTT:kill9/12/2021  -0.281632   0.219939  -1.281  0.20060    
TRTT:kill14/12/2021 -0.019215   0.219298  -0.088  0.93019    
TRTT:kill15/12/2021  0.170436   0.220992   0.771  0.44071    
TRTT:kill16/12/2021 -0.063750   0.219564  -0.290  0.77160    
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 1.216 on 1313 degrees of freedom
Multiple R-squared:  0.8204, Adjusted R-squared:  0.8191 
F-statistic: 599.9 on 10 and 1313 DF,  p-value: < 2.2e-16

Response: HCW
             Df Sum Sq Mean Sq   F value    Pr(>F)    
TRT           1  102.0   102.0   69.0252 2.396e-16 ***
kill          4  996.7   249.2  168.6297 < 2.2e-16 ***
Pre.Rinsed    1 7758.7  7758.7 5250.8209 < 2.2e-16 ***
TRT:kill      4    7.2     1.8    1.2135    0.3032    
Residuals  1313 1940.1     1.5                        
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1


 TRT emmean      SE   df lower.CL upper.CL
 NT   21.97 0.04768 1313    21.88    22.07
 T    22.65 0.04728 1313    22.56    22.74

Results are averaged over the levels of: kill 
Confidence level used: 0.95
```

9.2.2 Cold Carcase Weight

Cold Weight Model fitted with pre-rinsed weight and kill

```
Residuals:
    Min      1Q  Median      3Q     Max
-3.3273 -0.7160 -0.0094  0.6701  3.2055

Coefficients:
                    Estimate Std. Error t value Pr(>|t|)
(Intercept)          1.16427    0.60553   1.923  0.05536 .
TRTT                 0.38115    0.21685   1.758  0.07971 .
kill9/12/2021       -0.79888    0.26142  -3.056  0.00242 **
kill14/12/2021      -0.58214    0.26080  -2.232  0.02627 *
kill15/12/2021      -0.20854    0.25872  -0.806  0.42078
kill16/12/2021      -0.16784    0.26342  -0.637  0.52446
Pre.Rinsed           0.60413    0.01644  36.740  < 2e-16 ***
TRTT:kill9/12/2021   0.21998    0.34474   0.638  0.52383
TRTT:kill14/12/2021  0.71074    0.36764   1.933  0.05405 .
TRTT:kill15/12/2021  0.45915    0.37086   1.238  0.21656
TRTT:kill16/12/2021  0.21705    0.36761   0.590  0.55528
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 1.11 on 335 degrees of freedom
  (978 observations deleted due to missingness)
Multiple R-squared:  0.8187, Adjusted R-squared:  0.8132
F-statistic: 151.2 on 10 and 335 DF,  p-value: < 2.2e-16

Response: CW
            Df  Sum Sq Mean Sq   F value    Pr(>F)
TRT          1   15.69   15.69   12.7419 0.0004098 ***
kill         4  169.67   42.42   34.4437 < 2.2e-16 ***
Pre.Rinsed   1 1671.96 1671.96 1357.6475 < 2.2e-16 ***
TRT:kill     4    5.18    1.30    1.0524 0.3801725
Residuals  335  412.56    1.23
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1


 TRT emmean     SE  df lower.CL upper.CL
 NT    21.7 0.0898 335     21.5     21.8
 T     22.4 0.0858 335     22.2     22.5

Results are averaged over the levels of: kill
Confidence level used: 0.95
```

9.2.3 Chiller Shrink

Chiller Shrink model adjusted for Kill Date

```
Residuals:
     Min       1Q   Median       3Q      Max
-1.82310 -0.36585 -0.00387  0.38160  2.17312

Coefficients:
                     Estimate Std. Error t value Pr(>|t|)
(Intercept)          3.096690   0.309424  10.008  < 2e-16 ***
TRTT                 0.073209   0.110807   0.661   0.5093
kill9/12/2021       -0.130195   0.133584  -0.975   0.3304
kill14/12/2021       0.021877   0.133269   0.164   0.8697
kill15/12/2021       0.111307   0.132204   0.842   0.4004
kill16/12/2021      -0.692069   0.134608  -5.141 4.65e-07 ***
Pre.Rinsed          -0.013615   0.008402  -1.620   0.1061
TRTT:kill9/12/2021   0.051922   0.176158   0.295   0.7684
TRTT:kill14/12/2021  0.260112   0.187862   1.385   0.1671
TRTT:kill15/12/2021 -0.419459   0.189508  -2.213   0.0275 *
TRTT:kill16/12/2021  0.317823   0.187845   1.692   0.0916 .
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.5671 on 335 degrees of freedom
  (978 observations deleted due to missingness)
Multiple R-squared:  0.1557, Adjusted R-squared:  0.1305
F-statistic: 6.177 on 10 and 335 DF,  p-value: 1.141e-08

Analysis of Variance Table

Response: Shrink
            Df  Sum Sq Mean Sq F value    Pr(>F)
TRT          1   1.123  1.1234  3.4936  0.062480 .
kill         4  13.539  3.3848 10.5258 4.813e-08 ***
Pre.Rinsed   1   0.535  0.5354  1.6649  0.197834
TRT:kill     4   4.667  1.1666  3.6280  0.006543 **
Residuals  335 107.725  0.3216
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

 TRT emmean     SE  df lower.CL upper.CL
 NT    2.49 0.0459 335     2.40     2.58
 T     2.60 0.0438 335     2.52     2.69

Results are averaged over the levels of: kill
Confidence level used: 0.95
```

9.2.4 Purge

Purge Loss model fitted with Kill Date

```
Residuals:
     Min       1Q   Median       3Q      Max 
-0.70606 -0.14139  0.00254  0.13684  0.40095 

Coefficients:
                    Estimate Std. Error t value Pr(>|t|)    
(Intercept)          0.87837    0.06002  14.635   <2e-16 ***
TRTT                 0.09223    0.08488   1.087   0.2812    
kill9/12/2021        0.01087    0.08488   0.128   0.8985    
kill15/12/2021       0.03501    0.08488   0.412   0.6813    
TRTT:kill9/12/2021  -0.10299    0.12003  -0.858   0.3940    
TRTT:kill15/12/2021 -0.26591    0.12003  -2.215   0.0302 *  
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.2079 on 66 degrees of freedom
  (1 observation deleted due to missingness)
Multiple R-squared:  0.109,  Adjusted R-squared:  0.04149 
F-statistic: 1.615 on 5 and 66 DF,  p-value: 0.1684

Analysis of Variance Table

Response: Purge.Loss
          Df  Sum Sq  Mean Sq F value  Pr(>F)  
TRT        1 0.01700 0.017004  0.3934 0.53268  
kill       2 0.11624 0.058119  1.3446 0.26770  
TRT:kill   2 0.21571 0.107857  2.4953 0.09023 .
Residuals 66 2.85282 0.043224                  
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

 TRT emmean     SE df lower.CL upper.CL
 NT   0.894 0.0347 66    0.824    0.963
 T    0.863 0.0347 66    0.794    0.932

Results are averaged over the levels of: kill 
Confidence level used: 0.95
```

9.2.5 pH Decline

pH #1

```
REML criterion at convergence: -191.6

Scaled residuals: 
     Min       1Q   Median       3Q      Max 
-2.68299 -0.72028  0.04542  0.61176  2.58657 

Random effects:
 Groups   Name        Variance Std.Dev.
 kill     (Intercept) 0.005732 0.07571 
 Residual             0.020164 0.14200 
```

```
Number of obs: 198, groups:  kill, 4

Fixed effects:
             Estimate Std. Error        df t value Pr(>|t|)    
(Intercept)   6.94690    0.04043   3.40195 171.817 8.04e-08 ***
TRTT         -0.20173    0.02018 193.00218  -9.994  < 2e-16 ***
---
Signif. Codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Correlation of Fixed Effects:
     (Intr)
TRTT -0.247

 TRT emmean     SE   df lower.CL upper.CL .group
 T     6.75 0.0405 3.42     6.62     6.87  1    
 NT    6.95 0.0404 3.40     6.83     7.07   2   

Degrees-of-freedom method: kenward-roger 
Confidence level used: 0.95 
significance level used: alpha = 0.05
```

pH #2

```
REML criterion at convergence: -143.4

Scaled residuals: 
     Min       1Q   Median       3Q      Max 
-2.31896 -0.69421 -0.06138  0.65297  2.58563 

Random effects:
 Groups   Name        Variance Std.Dev.
 kill     (Intercept) 0.003948 0.06283 
 Residual             0.026015 0.16129 
Number of obs: 198, groups:  kill, 4

Fixed effects:
             Estimate Std. Error        df t value Pr(>|t|)    
(Intercept)   6.57970    0.03531   3.72298 186.317 1.58e-08 ***
TRTT         -0.12150    0.02293 192.99930  -5.299 3.15e-07 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Correlation of Fixed Effects:
     (Intr)
TRTT -0.321

 TRT emmean     SE   df lower.CL upper.CL .group
 T     6.46 0.0354 3.76     6.36     6.56  1    
 NT    6.58 0.0353 3.73     6.48     6.68   2   

Degrees-of-freedom method: kenward-roger 
Confidence level used: 0.95 
significance level used: alpha = 0.05
```

pH #3

```
REML criterion at convergence: -107.3

Scaled residuals: 
    Min      1Q  Median      3Q     Max 
-2.6954 -0.6029 -0.1226  0.6410  2.5869 

Random effects:
 Groups   Name        Variance Std.Dev.
 kill     (Intercept) 0.007219 0.08497 
 Residual             0.031090 0.17632 
Number of obs: 198, groups:  kill, 4

Fixed effects:
             Estimate Std. Error        df t value Pr(>|t|)    
(Intercept)   6.40500    0.04600   3.48397 139.249 1.19e-07 ***
TRTT         -0.08828    0.02506 192.99916  -3.522 0.000534 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Correlation of Fixed Effects:
     (Intr)
TRTT -0.270

 TRT emmean     SE   df lower.CL upper.CL .group
 T     6.32 0.0461 3.51     6.18     6.45  1    
 NT    6.41 0.0460 3.49     6.27     6.54   2   

Degrees-of-freedom method: kenward-roger 
Confidence level used: 0.95 
significance level used: alpha = 0.05
```

pH #4

```
REML criterion at convergence: -106.6

Scaled residuals: 
    Min      1Q  Median      3Q     Max 
-2.2872 -0.6605 -0.1558  0.7640  3.4825 

Random effects:
 Groups   Name        Variance Std.Dev.
 kill     (Intercept) 0.007072 0.08409 
 Residual             0.031213 0.17667 
Number of obs: 198, groups:  kill, 4

Fixed effects:
             Estimate Std. Error        df t value Pr(>|t|)    
(Intercept)   6.27180    0.04561   3.49551 137.517 1.19e-07 ***
TRTT         -0.04111    0.02511 192.99920  -1.637    0.103    
---
Signif. Codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Correlation of Fixed Effects:
     (Intr)
TRTT -0.273
```

```
 TRT emmean     SE   df lower.CL upper.CL .group
 T     6.23 0.0457 3.52     6.10     6.36  1
 NT    6.27 0.0456 3.50     6.14     6.41  1

Degrees-of-freedom method: kenward-roger
Confidence level used: 0.95
significance level used: alpha = 0.05
```

pH #5

```
REML criterion at convergence: -86.4

Scaled residuals:
    Min      1Q  Median      3Q     Max
-2.0149 -0.6723 -0.0117  0.6069  3.4826

Random effects:
 Groups   Name        Variance Std.Dev.
 kill     (Intercept) 0.009832 0.09916
 Residual             0.034490 0.18571
Number of obs: 198, groups:  kill, 4

Fixed effects:
             Estimate Std. Error        df t value Pr(>|t|)
(Intercept)   6.19460    0.05294   3.39845 117.005 3.01e-07 ***
TRTT         -0.02288    0.02640 193.00001  -0.867    0.387
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Correlation of Fixed Effects:
     (Intr)
TRTT -0.247

 TRT emmean     SE   df lower.CL upper.CL .group
 T     6.17 0.0530 3.42     6.01     6.33  1
 NT    6.19 0.0529 3.40     6.04     6.35  1

Degrees-of-freedom method: kenward-roger
Confidence level used: 0.95
significance level used: alpha = 0.05
```

pH #6

```
REML criterion at convergence: -18

Scaled residuals:
     Min       1Q   Median       3Q      Max
-2.07440 -0.64201 -0.09589  0.57553  2.71012

Random effects:
 Groups   Name        Variance Std.Dev.
 kill     (Intercept) 0.01051  0.1025
 Residual             0.04908  0.2215
Number of obs: 198, groups:  kill, 4
```

```
Fixed effects:
             Estimate Std. Error        df t value Pr(>|t|)    
(Intercept)   6.13160    0.05584   3.52335 109.806 2.35e-07 ***
TRTT         -0.04099    0.03149 192.99957  -1.302    0.195    
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Correlation of Fixed Effects:
     (Intr)
TRTT -0.279

 TRT emmean     SE   df lower.CL upper.CL .group
 T     6.09 0.0559 3.55     5.93     6.25  1    
 NT    6.13 0.0558 3.53     5.97     6.30  1    

Degrees-of-freedom method: kenward-roger 
Confidence level used: 0.95 
significance level used: alpha = 0.05 
```

pH 24

```
REML criterion at convergence: -145.2

Scaled residuals: 
    Min      1Q  Median      3Q     Max 
-1.6608 -0.5969 -0.1801  0.3634  5.0649 

Random effects:
 Groups   Name        Variance Std.Dev.
 kill     (Intercept) 0.003926 0.06266 
 Residual             0.021292 0.14592 
Number of obs: 160, groups:  kill, 4

Fixed effects:
             Estimate Std. Error        df t value Pr(>|t|)    
(Intercept)   5.69538    0.03539   2.92664 160.942 7.19e-07 ***
TRTT          0.03396    0.02368 157.92534   1.434    0.154    
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Correlation of Fixed Effects:
     (Intr)
TRTT -0.266

 TRT emmean     SE   df lower.CL upper.CL .group
 NT    5.70 0.0355 3.40     5.59     5.80  1    
 T     5.73 0.0375 3.87     5.62     5.83  1    
```