Final report prepared for MPSC Inc.

Shelf life and quality of Rinse&Chill lamb meat in extended chilled storage

Minh Ha, PhD; Rozita Vaskoska, MSc; and Robyn Warner, PhD

School of Agriculture and Food, Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Parkville, VIC 3010

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Executive summary

This study compared shelf life of loins and topsides collected from lamb carcasses either treated with Rinse&Chill® technology or control (no Rinse&Chill). Loins and topsides were collected from 48 carcasses (24 Rinse&Chill and 24 control). The initial weight and pH of the muscles were measured at 1-day post slaughter, while final pH, lipid oxidation, protein oxidation, colour, purge loss and bacterial counts were measured at days 0, 60, 70, 85, 95, 105, 115 and 140.

The initial weight of the loins and topsides was similar between the Rinse&Chill and control carcasses. The initial pH of most of the carcasses were higher than 5.7, indicating a high incidence of dark cutting in both carcass treatments. While the initial pH was slightly higher in Rinse&Chill loins and topsides, the final pH of the loins was lower in the muscles from Rinse&Chill carcasses and similar for topsides. Both loins and topsides from Rinse&Chill had a higher yield (less purge loss) up to 105 days of storage. The loins from Rinse&Chill carcasses had greater purge loss at 115 and 140 days compared to the control.

The total heme content, oxymyoglobin and metmyolobin were similar in the Rinse&Chill and control carcasses, suggesting similar level of protein oxidation. The muscles from Rinse&Chill carcasses had greater lightness and redness. The hue angle of the loins and the chroma of the topsides tended to be greater in the Rinse&Chill carcasses. Together, these colour parameters suggest Rinse&Chill may help reducing the undesirable dark colour of the meat.

Lipid oxidation, which affects flavour of meat, occurred to the same extent in the loins of the Rinse&Chill carcasses and the control. Lipid oxidation in the topsides from Rinse&Chill carcasses was slightly elevated, however, was well under the recommended acceptability threshold. In relation to storage time, there was little difference in lipid oxidation at most of the time points. These results indicate flavour of extended storage would unlikely be affected by lipid oxidation.

The standard plate count (SPC) was better in Rinse&Chill carcasses compared to the control up to 140 days. Coliforms showed a lower prevalence and concentration in loins in Rinse&Chill carcasses, and similar in topsides compared to the control. *Enterobacteriaceae* were similarly prevalent and present in the loins from Rinse&Chill and control carcasses, while they appeared to be more prevalent and present in the topsides from Rinse&Chill carcasses. *Salmonella* and *E. coli* bacteria were not detected in either treatment groups.

In conclusion, application of the Rinse&Chill® technology resulted in improved colour characteristics and overall microbiological quality during extended chilled storage. Additionally, yield improved for Rinse&Chill loins up to 105 days. Therefore, Rinse&Chill® technology is recommended to be applied to lamb carcasses to improve colour and microbiological quality up to 140 days and yield up to 105 days. While instrumental measurements indicate stable shelf life of lamb in extended storage up to 140 days, sensory assessment are needed to confirm odour acceptability and eating quality.

Introduction

With the globalization of the meat market, the export of meat is an important component for the Australian meat sector. Australia has 38 % share of the world sheep exports, of which 26 % is chilled lamb with an overall value of 36 % of the export. Asia and the Middle East are the key markets for Australian sheepmeat. The meat that is exported to distant destination should sustain its quality during the transport and fulfill the customer requirements at the destination. Transportation might take longer, in circumstances where the speed of transport is limited due to efforts for emission reduction for sustainability reasons (Mills et al., 2014). In addition, customers in the distant markets might set their own requirements for quality, as it has been the case of Japanese retail chains requesting lamb to have lower microbial count than 500 000 cfu/g (5.7 log cfu/g) (MLA, 2016). Therefore, ensuring a long meat shelf- life becomes essential export requirement.

Shelf- life of 80 and 84 days has historically been reported for VPC lamb stored at -0.3°C and 0.5°C, respectively (Gill & Penney, 1985; Kiermeier et al., 2013). Nowadays, shelf- life of minimum 100 days for VPC beef and lamb has become easily attainable with good temperature control (MLA, 2016). The feasibility of extended shelf life of 140 days under controlled storage at -1°C has been proven for Vacuum Packed Chilled (VPC) beef (Frank et al., 2019). Duration of shelf- life is expected to differ between beef and lamb, as pH of lamb tends to be higher, and the amount of available glucose for microbial growth is also expected to be greater than in beef (Mills et al., 2014). The end of shelf life of meat occurs when the `meat becomes unfit for use, human consumption, or sale`(MLA, 2016), which mainly occurs mainly due to lipid and protein oxidation from biochemical perspective, and microbial growth from biological perspective (Bellés et al., 2017). Extensive lipid oxidation results in rancid odours and flavours, protein oxidation affects the myoglobin resulting in meat discolouration, while microbial growth can cause both discolouration and odour in the meat samples, rendering the meat undesirable or even unsafe for consumption if pathogens also emerge in the sample.

Rinse&Chill@ is a technology for vascular perfusion of an isotonic solution composed of carbohydrates (glucose, maltose) and phosphates into an animal carcass. The solution is infused post- exsanguination and applied using a patented technology (Crane, 2009). The rinsing facilitates the chilling of the carcass, i.e. temperature reduction which in line affect the pH decline process. This technology has been applied to lamb (Fowler et al., 2017; Li, 2019; Mickelson et al., 2018), beef (da Cunha Moreira et al., 2018), pork (Kethavath, 2019) and bison (Mickelson & Claus, 2016) carcasses and it has been shown to have a range of positive effects on meat eating quality. The potential protective effects of Rinse&Chill towards the lipid oxidation, meat colour and maintaining a better microbiological quality make it a suitable candidate as a strategy to improve and extend lamb meat shelf life.

Project objective

The objective of this project was to compare the muscle characteristics, lipid oxidation, heme content, colour, purge loss and microbiological quality of two muscles of distinct nature, the loin (*longissimus lumborum et thoracis*) and topside (*semimembranosus*), of carcasses treated with Rinse&Chill vascular perfusion to carcasses slaughtered conventionally (control without vascular perfusion) during vacuumed chilled storage (VCS) over period of 140 days. Hence, the aim of the project is investigating whether Rinse&Chill technology offers advantages to conventional carcass treatment in achieving an extended shelf- life of lamb.

Materials and methods

Muscle collection, pH and weight

A total of 60 lambs were selected by MPSC and slaughtered at Hardwicks (Kyneton, Victoria, Australia). Immediately after exsanguination, 30 carcasses were subjected to Rinse&Chill vascular perfusion treatment with isotonic solution (98.5 % water, balance: glucose, phosphates, maltose); while the other 30 carcasses were treated as control (No Rinse&Chill) (Figure 1). Loin (*longissimus lumborum et thoracis*) and topside (*semimembranosus*) muscles from both left and right sides of all 60 carcasses were collected the next day (1 day post-mortem). All lamb muscles were labelled and transported to The University of Melbourne (UoM) under refrigerated conditions.

Upon receival of the muscles at UoM, the weight of the muscles was measured. Additionally, the pH and temperature were measured using a pH meter (model WP 80, TPS Pty Ltd, Brisbane, Victoria Australia) equipped with IJ 44 electrode (Ionode Pty Ltd., Brisbane, Victoria Australia) and a temperature compensation probe. Muscles from 24 Rinse&Chill and 24 control carcasses were selected for cutup (Figure 1), excluding the muscles with the highest pHs. Each muscle was cut into 2 blocks and vacuum packed.. For each muscle type (loin and topside), each block was allocated to a storage time (0; 60; 70; 85; 95; 105; 115 or 140 days) using incomplete randomisation across positions 1-96 as illustrated in Figure 2, resulting in a replication of 12 carcasses for each time point. The storage conditions were 0.5°C without exposure to light. All cut-ups and samplings were conducted in a temperature (6°C) controlled room.

At each designated storage time point, the following analyses were conducted: pH, lipid oxidation (TBARS assay), colour shelf- life (instrumental colour CIE LAB measurement), total heme protein content assay, purge loss/yield (weight), and microbiological shelf life assessment (total viable count, *Enterobacteriaceae*, Coliform, *Escherichia coli*, and *Salmonella* spp.). The pH was measured as described above.

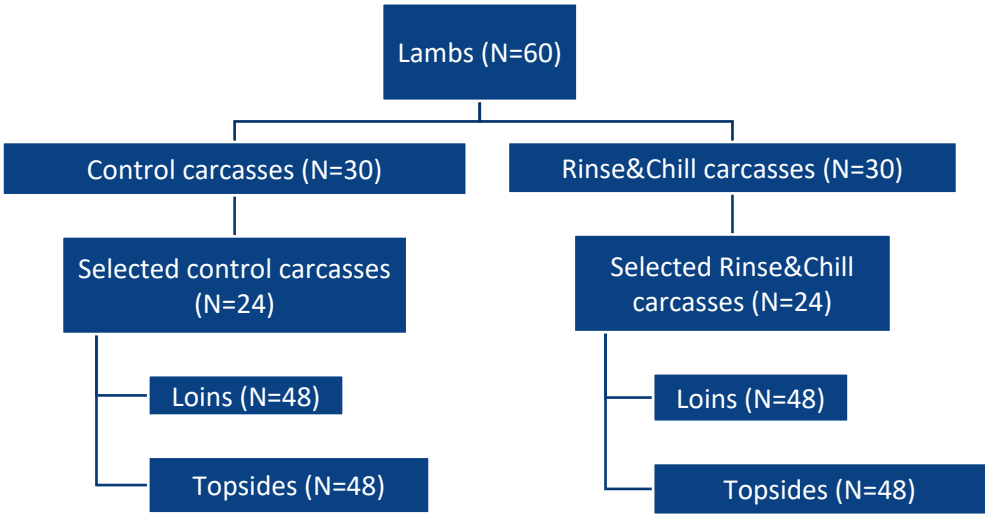


Figure 1. Diagram illustrating the carcass and muscle replication.

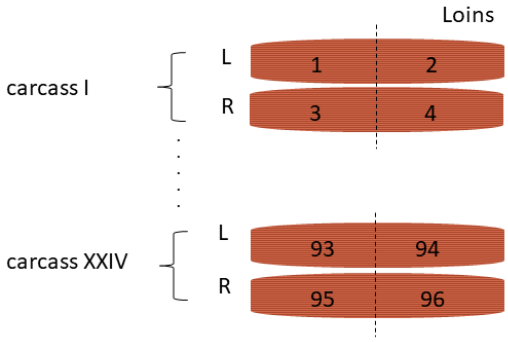


Figure 2. Diagram showing the storage period allocation to lamb muscles` positions. Storage times were randomized across positions 1-96. The procedure is illustrated for loins, and the same procedure applies for topsides.

Lipid oxidation

Lipid oxidation of the muscles was measured using the Thiobarbituric acid reactive substances assay (TBARS) method, modified from Jongberg et al. (2011). 10 g of frozen lamb sample were cut finely, after removing the connective tissue and visible fat. The sample was mixed with a 15 mL 10% solution of trichloroacetic acid and homogenized with a homogenizer with a 10mm probe (Ultra Turrax T25, IKA, Shaufen, Germany) at 13500 rpm, for 60 s. The mixture was filtered through a Whatman No.1 filter. A 1 ml sample (in duplicate) was mixed with 1 ml of 20 mM 2-thiobarbituric acid solution and placed in a water bath at 95°C for 55 minutes. The absorbance at 532 nm was measured with a spectrophotometer (Multiscan, Alicante, Spain) against a blank sample (500 µL water with 500 µL 20mM TBA). Results are expressed as mg MDA/kg meat using a standard curve with malonaldehyde.

Colour

The colour of the lamb muscles was measured using a Hunterlab (Miniscan EZ. Hunter Association Labs Inc., Virginia, USA), previously calibrated with black and white reference tiles. A D65 illuminant and 10° observer angle were used. CIE lightness (L*), redness (a*) and yellowness (b*) values were obtained as an average of two measurements, after removing the lamb from the vacuum bag and blooming for 30 min. Hue and chrome were calculated using the following formulas:

$$Hue = arctangent\,(\frac{b*}{a*})$$

$$Chroma = (a*^2 + b*^2)^{1/2}$$

The obtained value for hue in radians was further converted to degrees, using the respective degrees function in MS Excel (Microsoft, Redmond, Washington, USA).

Heme protein content

The heme protein content was measured using the protocol from (O'Brien et al., 1992). One g of lamb was homogenized in ice- cold Tris- HCl buffer (pH8) and rinsed with 1ml of the same buffer. A saturation with 75 % ammonium sulfate was performed to solubilize haemoglobin. After that, the samples were centrifuged for 10 min. at 5000 g (0°C). The supernatant was collected and mixed with 100 µM K3Fe(CN)6 as an oxidant. The heme concentration was measured by determination of the absorbance at 540, 580 and 420 nm with a spectrophotometer (Multiscan, Alicante, Spain). Coefficients of 3.19, 1.95 and 35.7 per gram per centimetre correspond to these wavelengths respectively for oxidized equine myoglobin.

Determination of myoglobin (deoxy-, oxy- and met-)

The redox forms of myoglobin were determined according to a modified method from Wu et al. (2016). One g of lamb was homogenized (Ultra Turrax T25, IKA, Shaufen, Germany) with 40 mM phosphate buffer (pH 6.8), followed by centrifugation of the homogenate at 5000 g for 30 min (4°C). The supernatant was collected and adjusted to volume of 5 ml using phosphate buffer. The proportions of the redox forms of myoglobin were determined using the absorbances of the supernatant measured at 503, 557 and 582 nm, using the following formulas:

$$DeoMb = \frac{C_{DeoMb}}{C_{Mb}} = -0.543R_1 + 1.594R_2 + 0.552R_3 - 1.329$$

$$OxyMb = \frac{C_{OxyMb}}{C_{Mb}} = 0.722R_1 - 1.432R_2 - 1.659R_3 + 2.599$$

$$OxyMb = \frac{C_{MetMb}}{C_{Mb}} = -0.159R_1 - 0.085R_2 + 1.262R_3 - 0.520$$

$$R_1 = \frac{A582}{A525},\ R_2 = \frac{A557}{A525},\ R_3 = \frac{A503}{A525}$$

Purge loss

The purge loss was calculated as the weight at each time point relative to the weight at 0 days storage (1 day post-mortem).

Microbiology

The microbiological shelf life was determined using measurements of Standard Plate Count (SPC) to determine the overall microbiological quality of the samples, indicator organisms (Coliforms, *Enterobacteriaceae* and *E. coli*) and *Salmonella spp*. as a pathogen. SPC was determined using the Petrifilm AOAC 990.12 method; *E.coli* was determined using the AOAC 991.14 method; Coliforms were determined using the AOAC 991.14 method; *Enterobacteriaceae* were determined using the AFNOR 3M 01/06 – 09/97 method; and *Salmonella* was determined using VIDAS AOAC 071101 using a sample of 25 g for detection.

Data analysis

The pH and weight data were analysed with the treatment and muscle as fixed factors, and carcass and side as random factors using Restricted Maximum Likelihood (REML) procedure in Genstat (version 18, VSN International, Hemel Hempstead, UK). REML was also used for analysis of TBARS, heme protein content, oxymyoglobin, deoxymyoglobin and metmyoglobin, L*, a*, b*, hue, chroma, purge loss and SPC. These variables were analysed using treatment, muscle type and days as fixed factors and carcass, side, muscles and position as random factors. The deoxymyoglobin data did not fulfil the assumption of normal distribution, therefore the data was transformed using log (base 10) transformation. *Enterobacteriaceae* and coliforms were represented as prevalence calculated as the number of samples with a detected presence divided by the total number of samples per treatment, as well as an average bacterial count (concentration) present in the positive samples. The bacterial growth, measured through the SPC, was modelled using the modified Gompertz equation (Zwietering et al., 1990):

$$\mathrm{Ln}\left(\frac{N}{No}\right) = A * \exp\{-exp\left[\frac{\mu * e}{A}(\lambda - t) + 1\right]\}$$

The model was fitted using the Solver function in MS Excel (Microsoft, Redmond, Washington, USA), using 5 iterations to minimize the residual sum of squares.

Results

Muscle characterization

The pH and weight of the loins and topsides 1-day post mortem are provided in Table 1. Overall, there was no statistically significant difference between the control and Rinse&Chill treated muscles in the initial weight (p(treatment)>0.05), however there was a significant two-way interaction between treatment and muscle type with the weight of the topside being reduced by 4.7 % compared to control (p(treatment x muscle type)<0.01) (Figure 3). There was no difference in the initial weight of the loin between the control and Rinse&Chill treatment (Figure 3).

Table 1. Descriptive statistics for initial pH and weight of lamb loin and topside from carcasses treated with Rinse&Chill compared to control (conventional) process.

	Muscle	Treatment	Mean	SD	Minimum	Maximum
Initial pH	Loin	Rinse&Chill	5.75	0.045	5.57	5.83
		Control	5.72	0.004	5.64	5.79
	Topside	Rinse&Chill	5.8	0.063	5.7	5.985
		Control	5.75	0.057	5.625	5.88
Initial weight (g)	Loin	Rinse&Chill	389.44	54.84	302	512
		Control	393.75	44.5	280	508
	Topside	Rinse&Chill	503.31	25.97	442	598
		Control	528.5	78.54	430	758

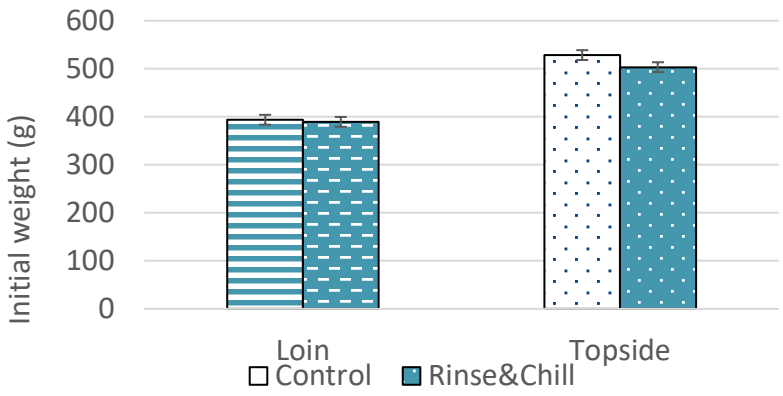


Figure 3. Initial weight of lamb loin and topside without (control) and with Rinse&Chill treatment. Main effects were as follows: p (treatment) =0.223; p (muscle type) <0.001, and interactive effects were as follows: p (treatment x muscle type) =0.009. Vertical error bars are least significant differences (lsd) for the interactive effect.

The initial pH (day 0 of storage) of the loin in both the control and Rinse&Chill was high, with an average pH of 5.72±0.004 and 5.75±0.045 (Table 1). Rinse&Chill muscles had a slightly higher pH than the control (0.03 units in loin, 0.05 units in topside) ($p<0.01$) (Figure 4). At most time points, the pH of the loin and topside were comparable (Supplementary data, Figure S1).

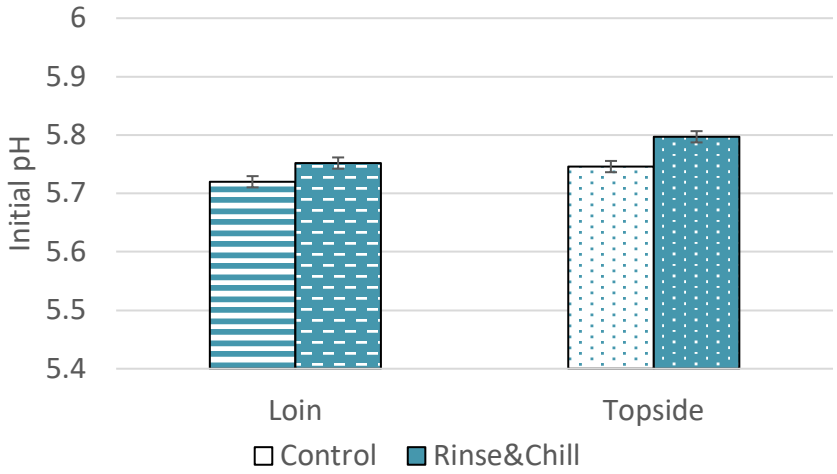
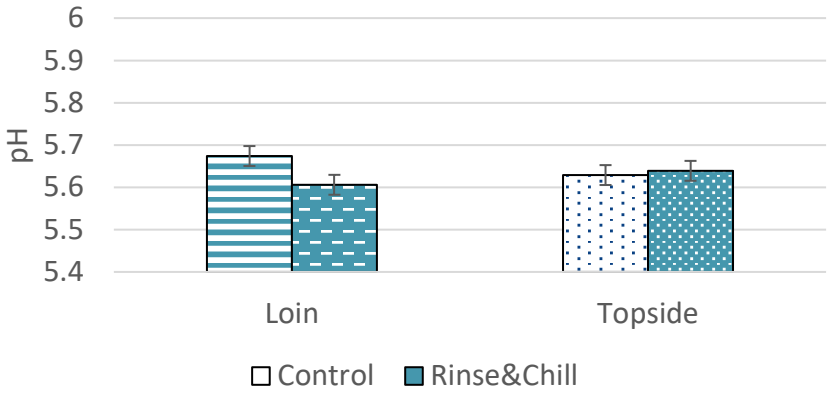
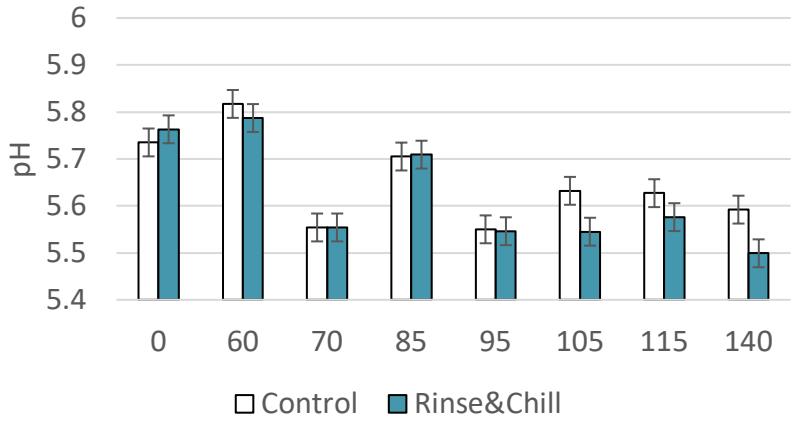


Figure 4. Initial pH (predicted means) of lamb loin and topside without (control) and with Rinse&Chill treatment. Main effects were as follows: p (treatment) <0.001; p (muscle type) <0.001, and interactive effects were as follows: p (treatment x muscle type) =0.007. Vertical error bars are least significant differences (lsd) for the interactive effect.

During storage, there was no significant difference in pH between the control and Rinse&Chill treatment (p(treatment)>0.05). However, there was an interactive effect between treatment and muscle types (p(treatment x muscle type) <0.01) on pH of the loin with Rinse&Chill carcasses, being 0.07 units lower compared to the pH of the control (Figure 5a). There was no difference in pH of topside between Rinse&Chill and control treatment (Figure 5a). The pH of the lamb muscles decreased with storage time (Figure 5b). In relation to storage time, there was a lower pH in Rinse&Chill muscles compared to the control on days 105 and 140 (Figure 5b). The three-way interaction between muscle type, treatment and days of storage on the pH levels was not significant (Table 2).



a)



b)

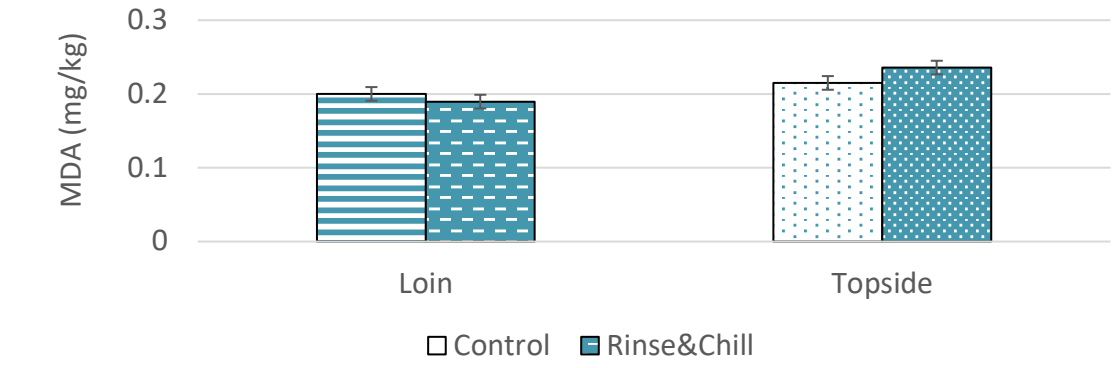
Figure 5. pH (predicted means) of lamb loin and topside without (control) and with Rinse&Chill treatment with storage (0-140 days). a) Interactive effect of Rinse&Chill and muscle type p (treatment x muscle type) =0.006; b) Interactive effect of Rinse&Chill and storage time p (treatment x days) =0.008. The levels of the significance of the main factors and the other interactive effects are provided in Table 2. Vertical error bars are least significant differences (lsd) for the interactive effect.

Table 2. pH (predicted means) of lamb loins and topsides from carcasses treated with Rinse&Chill or conventionally (control), during storage of 0-140 days.

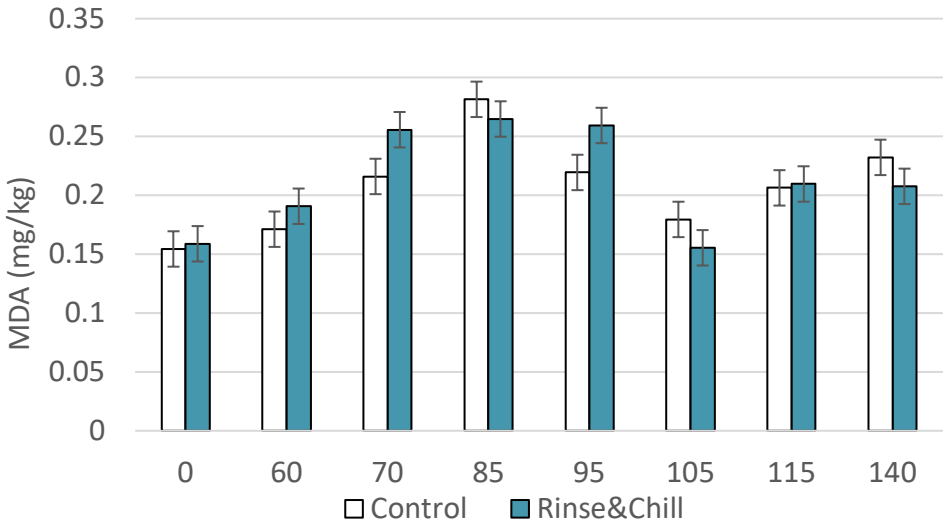
Muscle	Treatment	Storage (Days)								Level of significance (p)							SED (muscle x treatment x days)
		0	60	70	85	95	105	115	140	muscle	treatment	days	muscle x treatment	muscle x days	treatment x days	muscle x treatment x days	
Loin	Control	5.71	5.81	5.56	5.75	5.61	5.66	5.68	5.63	0.65	0.17	<0.001	0.006	0.008	0.008	0.089	0.043
	Rinse&Chill	5.73	5.79	5.48	5.70	5.50	5.55	5.58	5.51								
Topside	Control	5.76	5.82	5.55	5.67	5.49	5.61	5.57	5.55								
	Rinse&Chill	5.79	5.78	5.63	5.72	5.59	5.54	5.58	5.48								

Lipid oxidation during shelf- life

The MDA amount, as an indicator of lipid oxidation increased up to 85 days of storage, after which a decreasing trend was observed (Figure 6a). Overall, there was no significant difference between the Rinse&Chill and control muscles in their lipid oxidation (p(treatment)>0.05). In relation to the interaction between muscle type and treatment, a higher oxidation was observed in the topside of Rinse&Chill carcasses compared to the control, while there was no significant difference in the MDA amount of the loins (Figure 6a). At most storage periods, there was no significant difference between the control and the Rinse&Chill muscles, except at days 70 and 95, when a greater oxidation was observed in the Rinse&Chill muscles (p(treatment x days)=0.001) (Figure 6b), probably related to the observations in the topside (Figure 6a, Supplementary data, Figure S2). The three-way interaction between muscle type, treatment and days of storage on the TBARS levels was not significant (Table 3).



a)



b)

Figure 6. TBARS (predicted means) of lamb loin and topside without (control) and with Rinse&Chill treatment with storage (0-140 days). a) Interactive effect of Rinse&Chill and muscle type p (treatment x muscle type) =0.003; b) Interactive effect of Rinse&Chill and storage time p (treatment x days) =0.001). The levels of the significance of the main factors and the other interactive effects are provided in Table 3. Vertical error bars are least significant differences (lsd) for the interactive effect.

Table 3. TBARS (predicted means, mg MDA/ kg meat) of lamb loins and topsides from carcasses treated with Rinse&Chill or conventionally (control), during storage of 0-140 days.

Muscle	Treatment	Storage (Days)								Level of significance (p)							SED (muscle x treatment x days)
		0	60	70	85	95	105	115	140	muscle	treatment	days	muscle x treatment	muscle x days	treatment x days	muscle x treatment x days	
Loin	Control	0.15	0.15	0.19	0.30	0.21	0.16	0.20	0.23	<0.001	0.582	<0.001	0.003	<0.001	0.001	0.148	0.020
	Rinse&Chill	0.15	0.16	0.23	0.26	0.20	0.13	0.18	0.20								
Topside	Control	0.16	0.19	0.24	0.26	0.23	0.20	0.21	0.23								
	Rinse&Chill	0.17	0.22	0.29	0.27	0.31	0.18	0.24	0.21								

Colour change during shelf- life

The heme protein content was not affected by Rinse&Chill treatment (p>0.05). The heme protein content decreased with storage time with the topside having a greater heme protein content than the loin at all time points, except at 105 and 140 days when there was no difference between muscle types (p(muscle type x days)<0.001) (Figure 7). The three-way interaction between muscle type, treatment and days of storage on the total heme content was not significant (Table 4). The heme content was reduced by ~50 % with storage of 140 days.

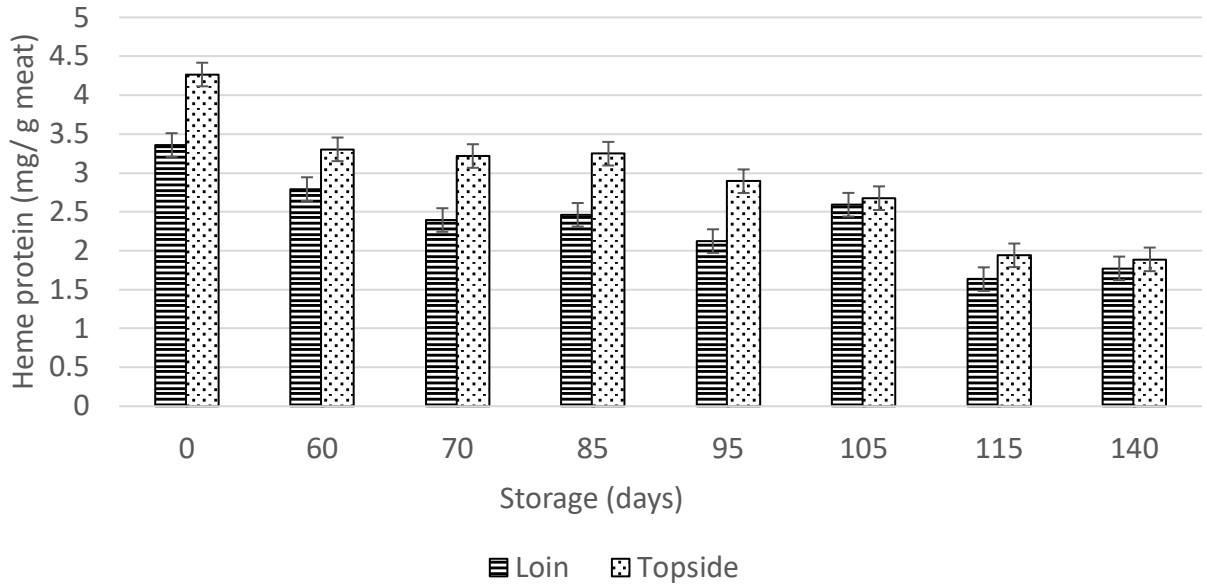


Figure 7. Heme protein content (predicted means) of lamb loin and topside with storage (0-140 days); p((muscle type x days) <0.001. The main effects were as follows: p (treatment) =0.462; p (days) <0.001; p (muscle type) <0.001. The levels of the significance of the main factors and the other interactive effects are provided in Table 4. Vertical error bars are least significant differences (lsd) for the interactive effect.

Table 4. Predicted means for total heme content (mg/g meat), oxymyoglobin (proportion), metmyoglobin (proportion) and deoxymyoglobin (proportion) of lamb loins and topsides, from carcasses treated with Rinse&Chill or conventionally (control), stored for 0-140 days.

Heme (mg/g meat)	Muscle	Treatment	Storage (Days)								Level of significance (p)							SED (muscle x treatment x days)
			0	60	70	85	95	105	115	140	muscle	treatment	days	muscle x treatment	muscle x days	treatment x days	muscle x treatment x days	
	Loin	Control	3.27	2.87	2.56	2.54	2.15	2.60	1.86	2.01	<0.001	0.462	<0.001	0.203	<0.001	0.074	0.815	0.231
		Rinse&Chill	3.45	2.72	2.23	2.38	2.10	2.59	1.41	1.54								
	Topside	Control	4.29	3.35	3.23	3.23	2.67	2.69	2.01	2.06								
		Rinse&Chill	4.25	3.26	3.21	3.27	3.11	2.66	1.87	1.72								

Oxymyoglobin	Muscle	Treatment	Storage (Days)								Level of significance (p)							SED (muscle x treatment x days)
			0	60	70	85	95	105	115	140	muscle	treatment	days	muscle x treatment	muscle x days	treatment x days	muscle x treatment x days	
	Loin	Control	0.60	0.37	0.35	0.35	0.23	0.39	0.18	0.16	0.138	0.813	<0.001	0.769	<0.001	0.259	0.809	0.061
		Rinse&Chill	0.52	0.39	0.28	0.36	0.21	0.41	0.19	0.20								
	Topside	Control	0.64	0.42	0.41	0.41	0.11	0.30	0.06	0.08								
		Rinse&Chill	0.51	0.43	0.41	0.45	0.19	0.26	0.09	0.06								

Metmyoglobin	Muscle	Treatment	Storage (Days)								Level of significance (p)							SED (muscle x treatment x days)
			0	60	70	85	95	105	115	140	muscle	treatment	days	muscle x treatment	muscle x days	treatment x days	muscle x treatment x days	
	Loin	Control	0.30	0.54	0.53	0.52	0.62	0.48	0.69	0.68	0.203	0.508	<0.001	0.684	0.004	0.365	0.642	0.057
		Rinse&Chill	0.37	0.51	0.59	0.52	0.65	0.47	0.66	0.65								
	Topside	Control	0.28	0.49	0.50	0.47	0.75	0.50	0.74	0.74								
		Rinse&Chill	0.40	0.48	0.50	0.44	0.70	0.62	0.73	0.76								

Deoxymyoglobin*	Muscle	Treatment	Storage (Days)								Level of significance (p)							SED (muscle x treatment x days)
			0	60	70	85	95	105	115	140	muscle	treatment	days	muscle x treatment	muscle x days	treatment x days	muscle x treatment x days	
	Loin	Control	-1.04 (0.09)	-1.06 (0.09)	-0.97 (0.11)	-0.93 (0.12)	-0.86 (0.14)	-0.98 (0.10)	-0.93 (0.12)	-0.82 (0.15)	0.889	0.486	<0.001	0.022	<0.001	0.091	0.486	0.060
		Rinse&Chill	-0.96 (0.11)	-1.03 (0.09)	-0.95 (0.11)	-0.93 (0.12)	-0.89 (0.13)	-0.97 (0.11)	-0.86 (0.14)	-0.85 (0.14)								
	Topside	Control	-1.14 (0.07)	-1.03 (0.09)	-1.09 (0.08)	-0.94 (0.11)	-0.87 (0.14)	-0.76 (0.18)	-0.73 (0.19)	-0.78 (0.17)								
		Rinse&Chill	-1.05 (0.09)	-1.10 (0.08)	-1.07 (0.08)	-1.02 (0.09)	-0.99 (0.10)	-0.94 (0.11)	-0.79 (0.16)	-0.77 (0.17)								

*Data for deoxymyoglobin were transformed (log 10). Values in parentheses are back-transformed data.

There were no significant differences between the Rinse&Chill and the control treatment in the presence of oxymyoglobin and metmyoglobin in the lamb muscles ($p>0.05$). Oxymyoglobin decreased, while metmyoglobin increased with storage time in lamb loins and topsides. At 70 days of storage, the topside had greater presence of oxymyoglobin than the loin, while at 105, 115 and 140 days the loin had higher oxymyoglobin than the topside (Figure 8). On the contrary, topside had greater amount of metmyoglobin than the loin at 85, 115 and 140 days of storage (Figure 9). On the remaining days, there was no difference between the muscles in the oxymyoglobin and metmyoglobin content (Figures 8 and 9). The three-way interaction between muscle type, treatment and days of storage on the presence of oxymyoglobin and metmyoglobin was not significant (Table 4).

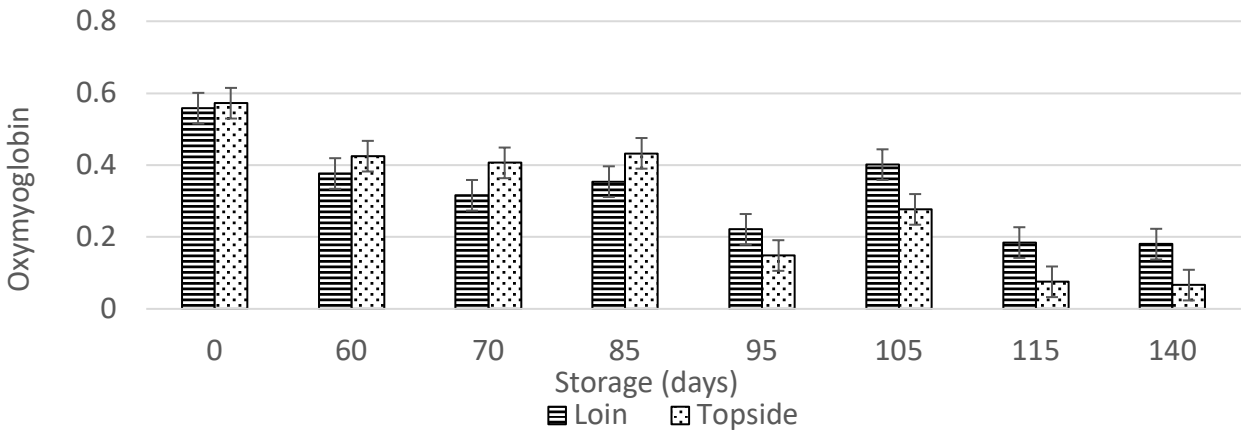


Figure 8. Oxymyoglobin proportion (predicted means) in lamb loin and topside with storage (0-140 days); p((muscle type x days) <0.001. The levels of the significance of the main factors and the other interactive effects are provided in Table 4. Vertical error bars are least significant differences (lsd) for the interactive effect.

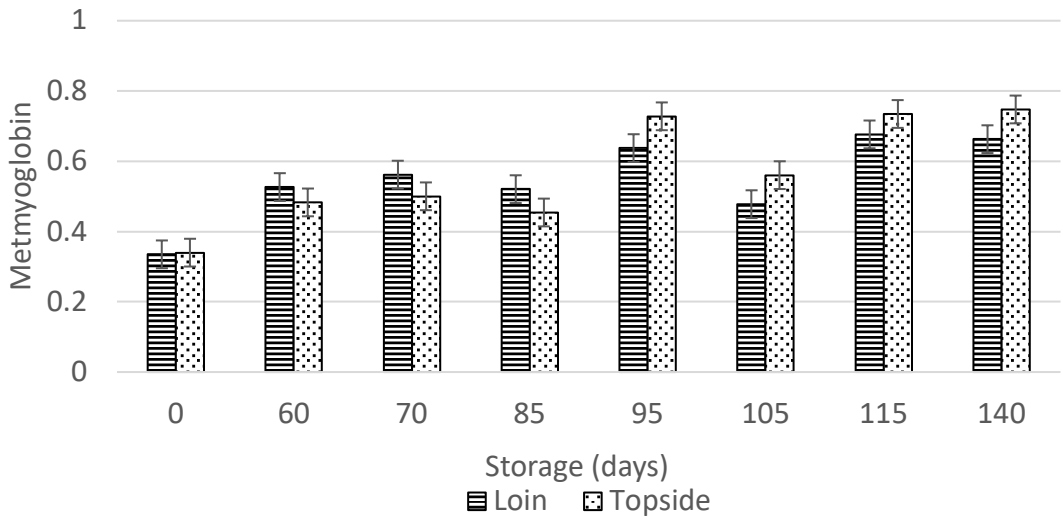


Figure 9. Metmyoglobin proportion (predicted means) in lamb loin and topside with storage (0-140 days); p((muscle type x days) =0.004. The levels of the significance of the main factors and the other interactive effects are provided in Table 4. Vertical error bars are least significant differences (lsd) for the interactive effect.

The deoxymyoglobin content increased with storage time (Table 4). Overall, there was no difference in the deoxymyoglobin presense in lamb muscles between the Rinse&Chill carcasses and the control across storage (p(treatment)>0.05). In relation to the muscle types, there was no difference in deoxymyoglobin presence in the loin muscle between Rinse&Chill and the control, however the deoxymyoglobin presence was lower in topsides from Rinse&Chill carcasses compared to the control (Figure 10). The three-way interaction between muscle type, treatment and days of storage on the presence of deoxymyoglobin was not significant (Table 4).

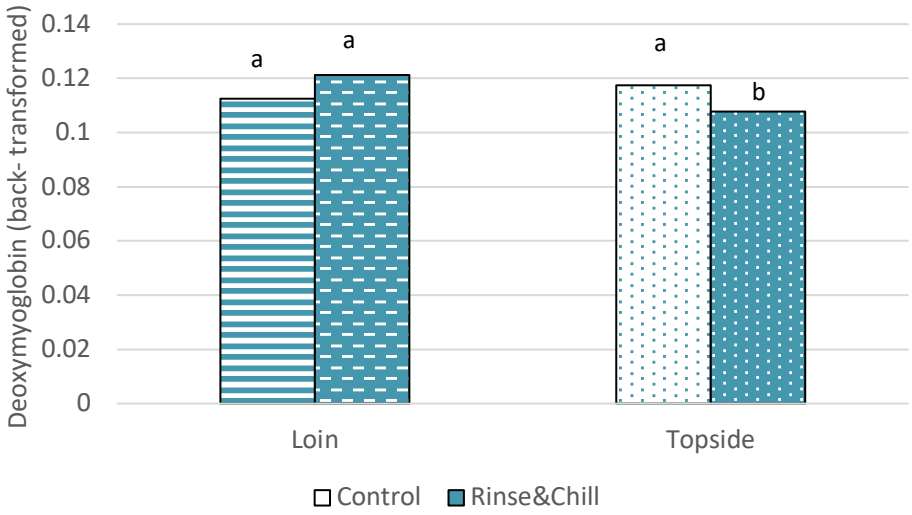


Figure 10. Deoxymyoglobin presence (back- transformed means) in lamb loin and topside without (control) and with Rinse&Chill treatment with storage (0-140 days). Interactive effect of Rinse&Chill and muscle type p (treatment x muscle type) =0.022. The levels of the significance of the main factors and the other interactive effects are provided in Table 4. Different letters mean significant difference.

In terms of colour, muscles from Rinse&Chill carcasses had a greater lightness compared to the control (Figure 11). The lightness showed a decreasing trend with storage time, independently of treatment (Supplementary data, Figure S3). The three-way interaction between muscle type, treatment and days of storage on lightness was not significant (Table 5).

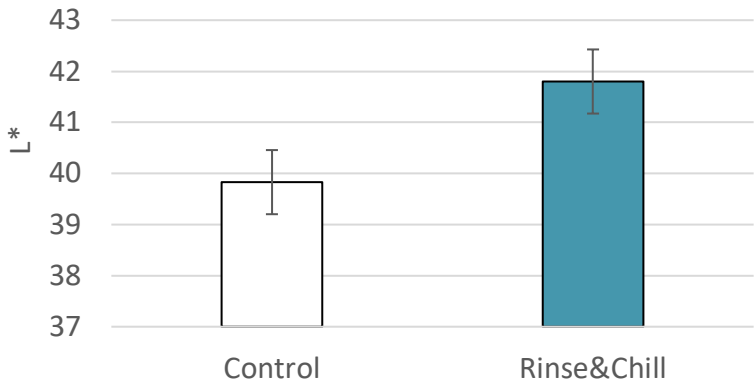


Figure 11. Lightness (L*) of Rinse&Chill and control lamb muscles during storage (0-140 days); p (treatment) =0.003. The levels of the significance of the main factors and the other interactive effects are provided in Table 5. Vertical error bars are least significant differences (lsd) for the interactive effect.

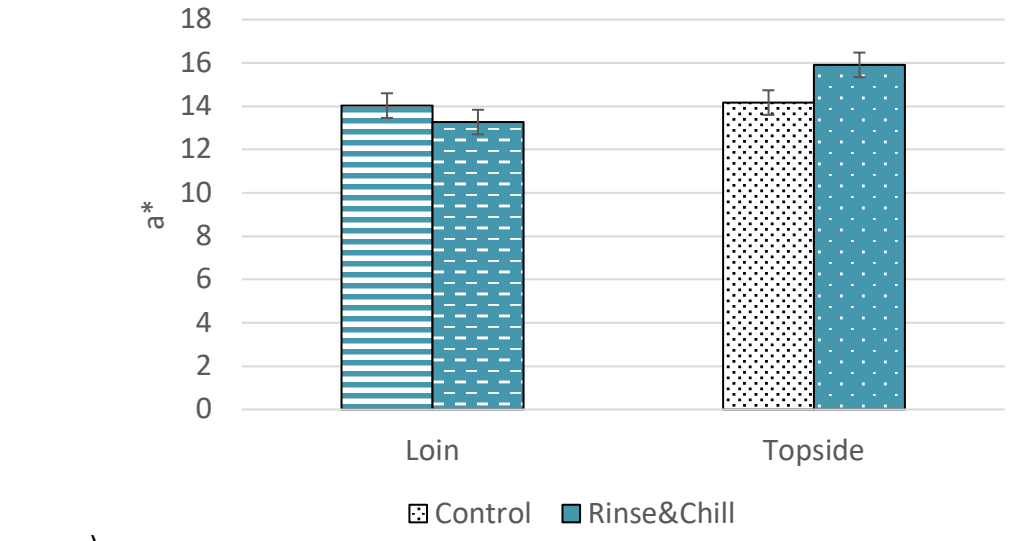
There was no overall difference between Rinse&Chill and the control in relation to redness, but topsides from carcasses treated with Rinse&Chill had higher redness than the control ($p<0.001$)(Figure 12a). There was no difference of the redness of the loin between Rinse&Chill and the control. In addition, lamb muscles from Rinse&Chill treated carcasses had higher redness at 60, 85 and 95 days of storage, while on the remaining days of storage there was no difference between the redness of Rinse&Chill and the control ($p<0.001$) (Figure 12b). The redness decreased with storage time, and it was greater in the lamb topside compared to the loin to 95 days of storage (Supplementary data, Figure S4). The three-way interaction between muscle type, treatment and days of storage on the muscle redness was not significant (Table 5).

Table 5. Lightness (L*), redness (a*) and hue (predicted means) of colour of lamb loins and topsides from carcasses treated with Rinse&Chill or conventionally (control), stored for 0-140 days.

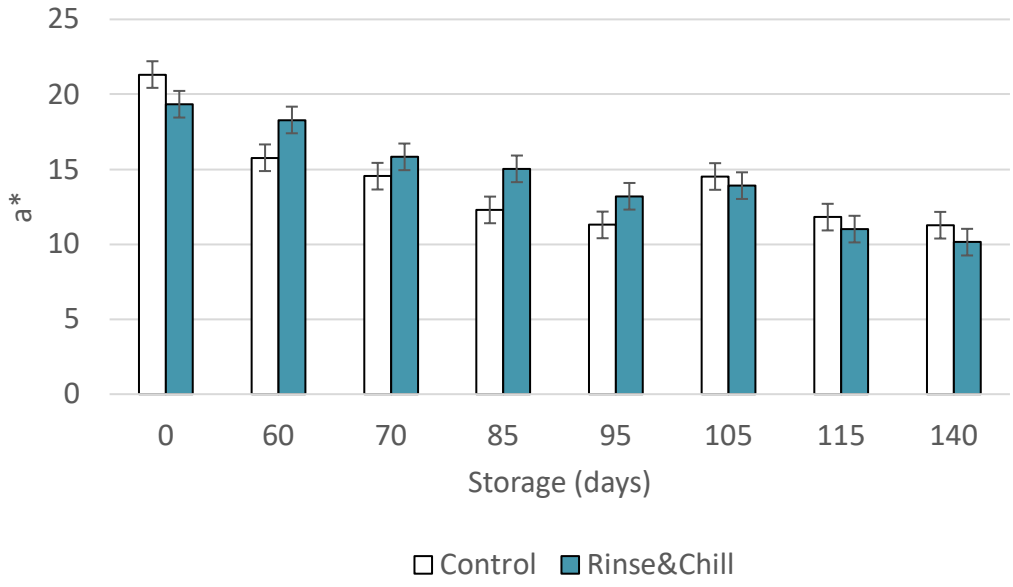
	Muscle	Treatment	Storage (Days)								Level of significance (p)							sed (muscle x treatment x days)
L*			0	60	70	85	95	105	115	140	muscle	treatment	days	muscle x treatment	muscle x days	treatment x days	muscle x treatment x days	
	Loin	Control	39.35	38.69	38.73	39.64	39.3	43.5	40.13	38.54	0.507	0.003	<0.001	0.239	0.166	0.942	0.378	1.408
		Rinse&Chill	41.81	40.94	42.79	41.37	39.64	47.44	41.64	41.29								
	Topside	Control	40.06	40.77	40.3	39.3	38.18	44.41	37.49	38.91								
		Rinse&Chill	39.79	43.36	40.8	41	40.67	45.98	40.03	40.24								

	Muscle	Treatment	Storage (Days)								Level of significance (p)							sed (muscle x treatment x days)
a*			0	60	70	85	95	105	115	140	muscle	treatment	days	muscle x treatment	muscle x days	treatment x days	muscle x treatment x days	
	Loin	Control	20.15	14.43	14.28	12.02	11.71	14.23	13.36	12.04	<0.001	0.324	<0.001	<0.001	<0.001	<0.001	0.061	1.275
		Rinse&Chill	18.44	15.55	13.57	13.39	10.34	13.66	10.43	10.8								
	Topside	Control	22.49	17.11	14.8	12.55	10.86	14.81	10.25	10.51								
		Rinse&Chill	20.25	21.02	18.09	16.67	16.05	14.17	11.6	9.47								

	Muscle	Treatment	Storage (Days)								Level of significance (p)							sed (muscle x treatment x days)
Hue			0	60	70	85	95	105	115	140	muscle	treatment	days	muscle x treatment	muscle x days	treatment x days	muscle x treatment x days	
	Loin	Control	28.59	43.05	44.03	46.44	47.98	46.5	46.82	49.18	0.008	0.242	<0.001	0.002	0.08	0.033	0.151	2.397
		Rinse&Chill	33.08	41.04	48.13	46.39	53.02	49.56	53.14	53.33								
	Topside	Control	28.25	40.14	42.69	46.76	49.26	45.95	50.63	51.38								
		Rinse&Chill	31.18	38.44	39.71	42.62	42.47	47.94	49.98	54.51								



a)



b)

Figure 12. Redness (a*, predicted means) of lamb loin and topside without (control) and with Rinse&Chill treatment with storage (0-140 days). a) Interactive effect of Rinse&Chill and muscle type p (treatment x muscle type) <0.001; b) Interactive effect of Rinse&Chill and storage time p (treatment x days) <0.001). The levels of the significance of the main factors and the other interactive effects are provided in Table 5. Vertical error bars are least significant differences (lsd) for the interactive effect.

There was a greater yellowness of the muscles from Rinse&Chill carcasses compared to the control (Figure 13). The yellowness (b*) of the Rinse and Chill loins on days 70, 85 and 140 was greater than the yellowness of the loins from the control treatment, while the topsides in Rinse&Chill carcasses had greater yellowness than the control at 60, 70, 85, 95 and 115 days of storage.

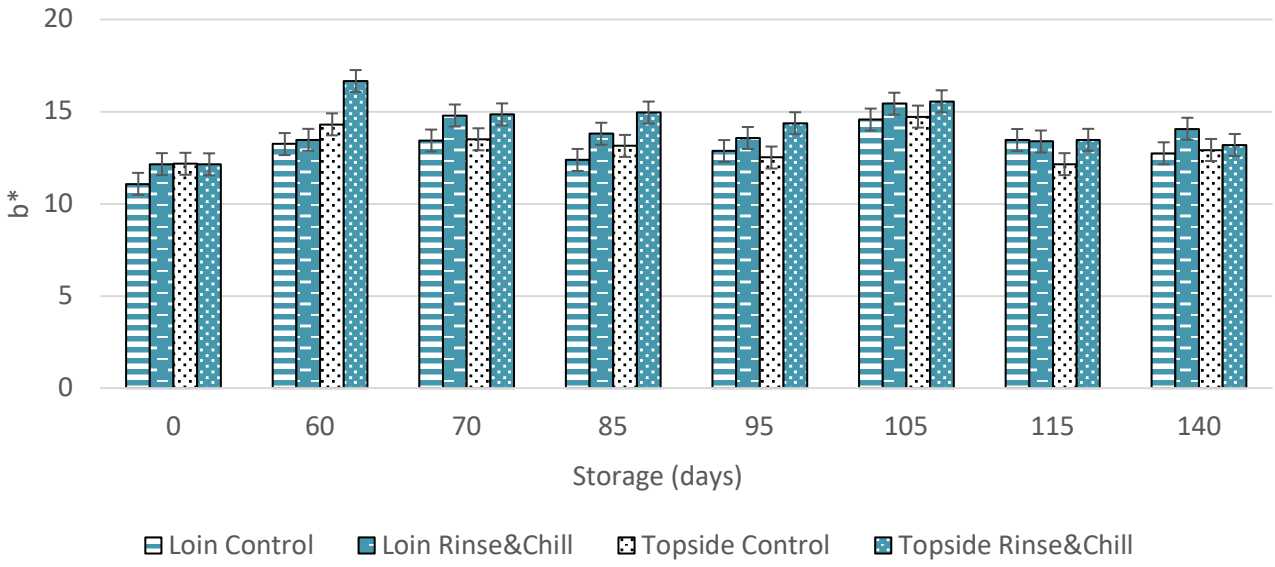
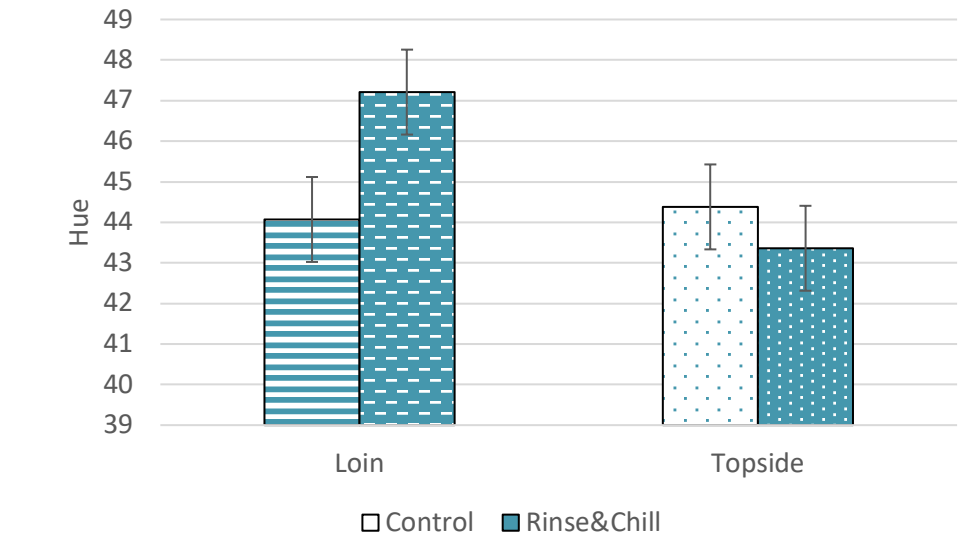
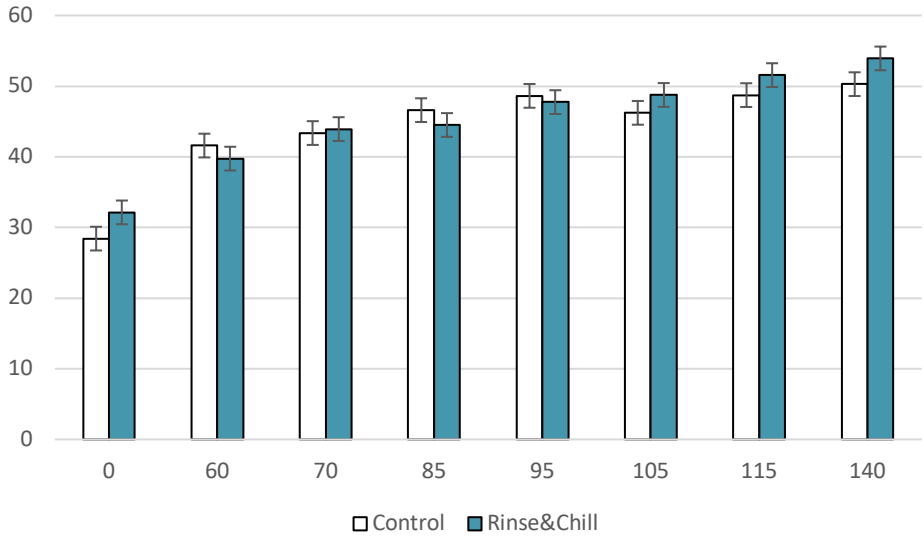


Figure 13. Yellowness (b*, predicted means) of lamb loins and topsides without (control) and with Rinse&Chill treatment with storage (0-140 days), interactive effect of Rinse&Chill, muscle type and storage days p (treatment x muscle type x days) =0.049. The levels of the significance of the main factors and the other interactive effects are provided in Table 5. Vertical error bars are least significant differences (lsd) for the interactive effect.

The hue of the muscles colour was not influenced by the treatment alone ($p>0.05$), but Rinse&Chill carcasses had higher hue of the loin colour (Figure 14a). There was no difference of the colour hue in the topsides between Rinse&Chill and the control (Figure 14b). In relation to storage time, Rinse&Chill muscles had greater colour hue at the first (day 0) and last day (day 140) of storage, while on the remaining days there was no significant difference between the treatments (Figure 14b). The hue increased with storage time (Figure 14b). The three-way interaction between muscle type, treatment and days of storage on the muscle colour hue was not significant (Table 5).



a)



b)

Figure 14. Hue (predicted means) of lamb loin and topside colour without (control) and with Rinse&Chill treatment with storage (0- 140 days). a) Interactive effect of Rinse&Chill and muscle type p (treatment x muscle type) =0.002. b) Interactive effect of Rinse&Chill and storage time p (treatment x days) =0.033. The levels of the significance of the main factors and the other interactive effects are provided in Table 5. Vertical error bars are least significant differences (lsd) for the interactive effect.

The colour chroma was greater in topsides from Rinse&Chill carcasses compared to the control on days 60, 70, 85 and 95, while there were no significant differences in the chroma of the loins due to treatment (Figure 15).

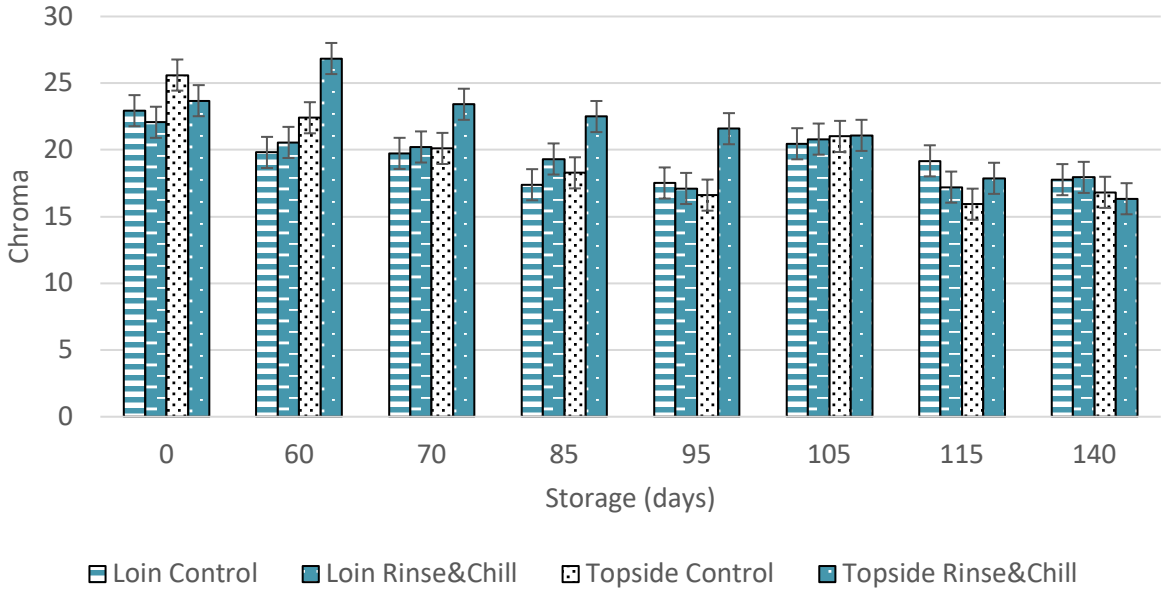


Figure 15. Colour chroma (predicted means) of lamb loins and topsides without (control) and with Rinse&Chill treatment with storage (0-140 days), interactive effect of Rinse&Chill, muscle type and storage days p (treatment x muscle type x days) =0.032. The main effects were as follows: p (treatment) =0.023; p (days) <0.001; p (muscle type) <0.001. The two- way interactions were as follows: p (muscle type x days) <0.001, p (treatment x days)<0.001, p (treatment x muscle type)=0.002.

Water- holding capacity

There was no significant difference between Rinse&Chill and control treatment in relation to purge loss overall, but there was an interactive effect of treatment, storage time and muscle type. On day 60, there was no difference between treatments (Figure 16). From days 60 to 105 Rinse&Chill muscles had lower purge loss than the control (Figure 16). At the last two time points (115 and 140 days), Rinse&Chill had higher purge loss than the control (Figure 16).

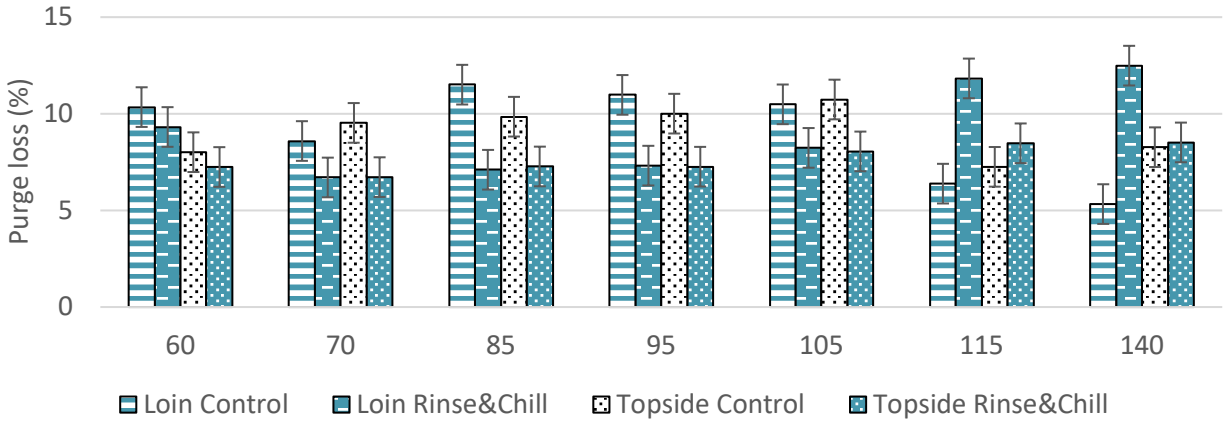
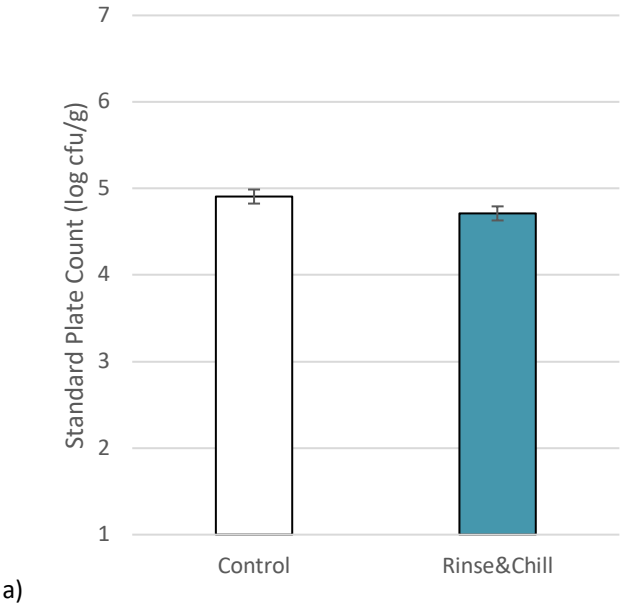


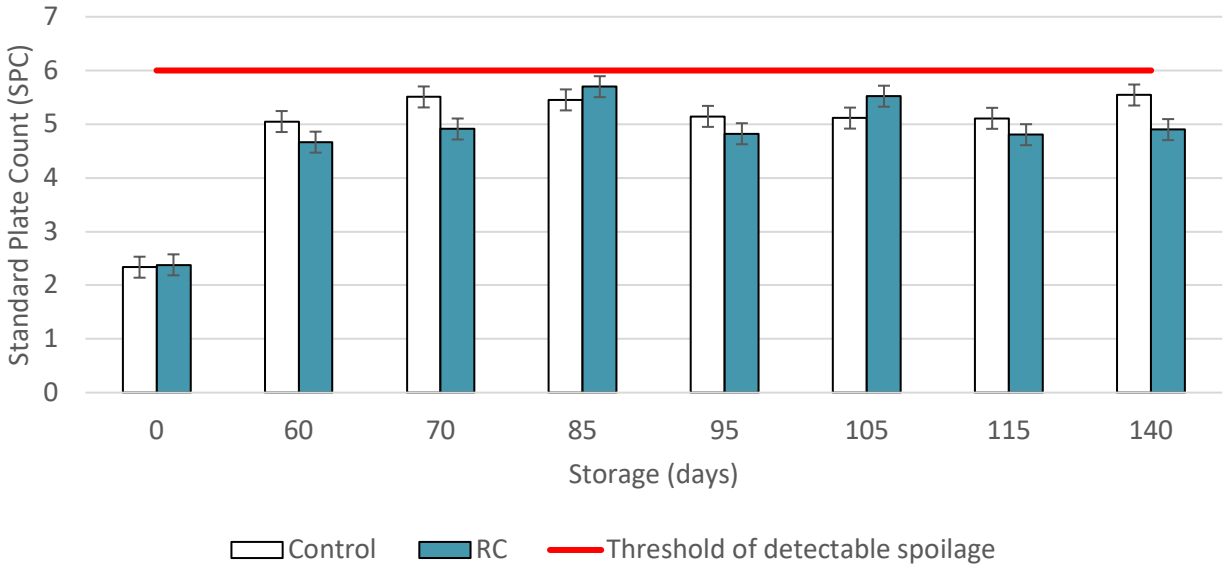
Figure 16. Purge loss (predicted means) of lamb loins and topsides without (control) and with Rinse&Chill treatment with storage (0-140 days), interactive effect of Rinse&Chill, muscle type and storage days p (treatment x muscle type x days) =0.045. The main effects were as follows: p (treatment) =0.166; p (days) <0.001; p (muscle type) =0.151. The two- way interactions were as follows: p (muscle type x days) =0.302, p (treatment x days)<0.001, p (treatment x muscle type)=0.011.

Microbiological shelf life

Rinse&Chill muscles had greater microbiological quality overall as visible from the lower SPC compared to the control (Figure 17a). In relation to storage time, Rinse&Chill muscles had lower SPC on most days of storage (60, 70, 115 and 140) except for storage of 105 days when Rinse&Chill muscles had greater SPC (Figure 17b). The frequency of samples with counts greater than 6 log cfu/g was lower in Rinse&Chill loins than in control loins (7 vs 2 samples), while in the topside the difference was smaller (4 vs 3 samples).



a)



b)

Figure 17. SPC (predicted means) of loin and topside without (control) and with Rinse&Chill treatment with storage (0-140 days). a) Effect of Rinse&Chill p (treatment) =0.002; b) Interactive effect of Rinse&Chill and storage time p (treatment x days) <0.001). The main effects were as follows: p (treatment) =0.002; p (days) <0.001; p (muscle type) =0.006. There was a significant interaction between muscle type and storage time p (muscle type x days) =0.390, while the two -way interaction between treatment and muscle type p(treatment x muscle type)=0.513 and the three-way interaction p (muscle type x treatment x days) =0.192 were not significant.

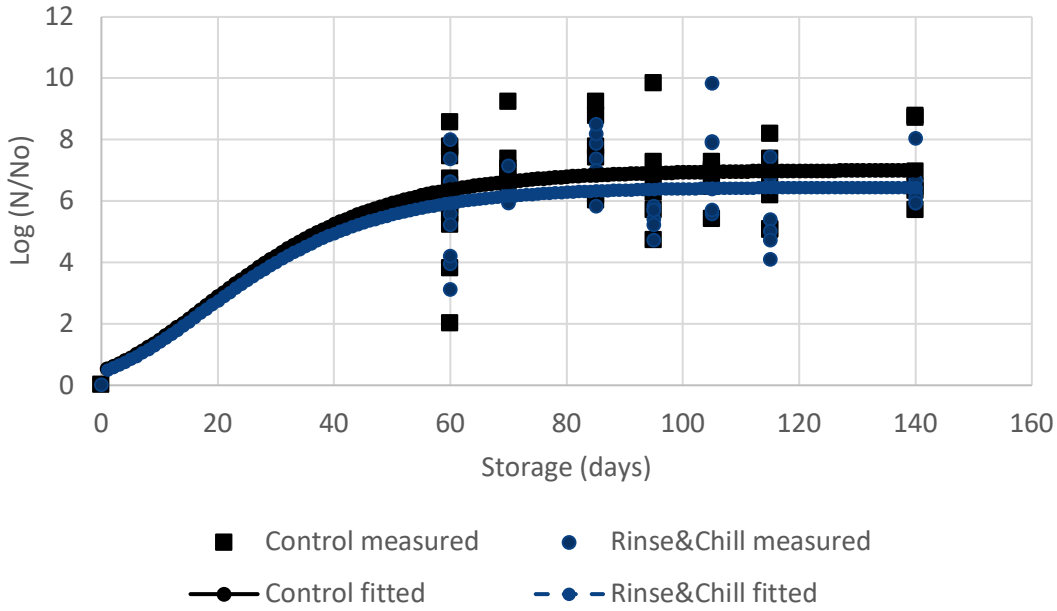
The modelling of the bacterial growth (Table 6, Figure 18), showed that the bacterial growth had different dynamics in the two lamb muscle types and in the two treatments (Rinse&Chill and control). Namely, the growth rate of bacteria was slower in loins from Rinse&Chill carcasses compared to the control, and the reached lower maximum level (Figure 18a). In the topsides, while the growth rate of bacterias was greater in Rinse&Chill carcasses the growth was modelled as very delayed (lag phase of 42 days) and also reaching a lower level compared to the control (Figure 18b, Table 6).

Table 6. Model parameters for modified Gompertz equation (Zwietering et al., 1990). A- asymptote (maximum level of growth, μ- growth rate, λ- lag phase (time to growth), RSS- residual sum of squares.

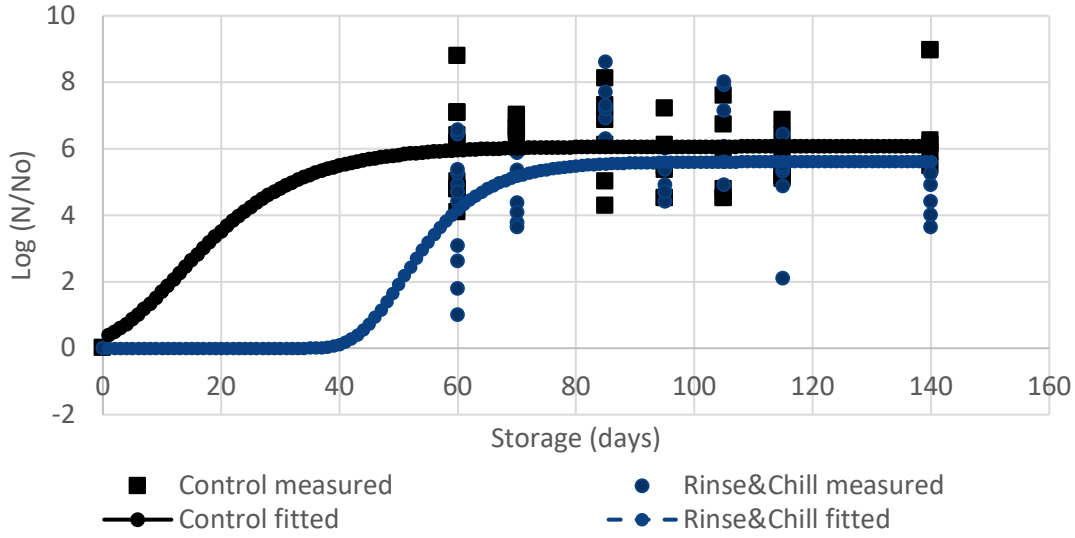
Muscle	**Treatment**	**A**	**μ**	**λ**	**RSS**
Loin	Control	7.002134	0.227682	0.001106	19.62979
Loin	Rinse&Chill	6.458466	0.218335	0.001106	16.04484
Topside	Control	6.061502	0.3	1.111858	14.63664
Topside	Rinse&Chill	5.610871	0.420174	42.78687	23.36422

Coliform bacteria and *Enterobacteriaceae* were not present on the first day of storage (Table 7). During storage, the prevalence and concentration of coliforms was lower in Rinse&Chill loins than in control loins (Table 7). The prevalence of *Enterobacteriaceae* in the loins was comparable between the control and the Rinse&Chill treatment (Table 7). As for the topsides, overall, the prevalence and concentration were also comparable between Rinse&Chill and control, while Rinse&Chill appeared to have higher prevalence and concentration of *Enterobacteriacea* compared to the control (Table 7). Coliforms did not show a consistent pattern with storage time, while *Enterobacteriaceae* were detected in 83-100% of the carcasses with storage of 105 days or more (Table 6). *E.coli* was not detected in any of the samples, and it was reported as <10 cfu/g. The loin had overall greater SPC than the topside (Supplementary data, Figure S5). *Salmonella* was also not detected (in 25 g samples) in any of the tested samples.

The presence of strong sour and off odours was detected in some samples, and that was reported in larger number of samples for Rinse&Chill compared to the control (Supplementary data, Figures S6). However, as these detected odours are not directly correlated to the SPC (Supplementary data, Figure S8), it is difficult to interpret them in the absence of sensory study. It is also emphasised that



a)



b)

Figure 18. SPC data for bacterial growth in lamb muscles from carcasses treated with Rinse&Chill and control, as a function of temperature with fitted modified Gompertz model (Zwietering et al., 1990). a) loins, b) topsides.

Table 7. Prevalence and concentration of coliform bacteria and *Enterobacteriaceae* in lamb loins and topsides from carcasses treated with Rinse&Chill compared to conventional (control) slaughter, during storage (0-140 days).

Days	Coliforms				*Enterobacteriaceae*			
	Control		Rinse&Chill		Control		Rinse&Chill	
	Loin							
	Prevalence	Mean log cfu/g	Prevalence	Mean log cfu/g	Prevalence	Mean log cfu/g	Prevalence	Mean log cfu/g
0	0.00		0.00		0.00		0.00	0
60	0.00		0.00		0.00		41.67	1.75
70	16.67	1.30	0.00		50.00	2.13	66.67	1.43
85	0.00		0.00		0.00		16.67	1.00
95	0.00		0.00		50.00	2.24	16.67	1.00
105	80.00	1.74	14.29	1.30	100.00	3.34	100.00	3.19
115	50.00	2.40	0.00		100.00	2.25	100.00	1.53
140	100.00	1.74	83.33	2.36	100.00	2.21	100.00	2.62
	Topside							
0	0.00		0.00		0.00		0.00	
60	50.00	1.80	33.33	1.94	58.33	2.00	33.33	1.87
70	0.00	0.00	33.33	1.91	50.00	1.53	50.00	1.89
85	33.33	1.42	83.33	1.78	66.67	1.75	83.33	1.87
95	0.00		0.00		0.00		16.67	1.00
105	16.67	1.00	16.67	2.36	83.33	1.74	100.00	2.59
115	0.00	0.00	0.00		100.00	1.99	83.33	2.59
140	16.67	1.48	0.00		83.33	1.63	100.00	2.09

Red-greater prevalence or concentration
Yellow=same prevalence or concentration
Green= lower prevalence or concentration

Discussion

In relation to the muscle characterization, the differences in the initial weight of the topside do not necessarily reflect the effect of the Rinse&Chill treatment, as it could be a systematic effect of the carcass weight due to the assigning of different carcasses to the treatment and not including the hot carcass weight in the analysis. Muscle pH is an important parameter that affects meat texture, water holding capacity as well as biochemical changes related to meat colour and oxidation. The average pH of both control and Rinse&Chill carcasses being higher than 5.7, corresponds to previous reports that pH of lamb tends to be higher than in beef with cuts often exceeding 5.8 (Mills et al., 2014). Our results of greater pH in Rinse&Chill compared to the control disagree with our expectations and previous reports where the ovine *longissimus thoracis* et *lumborum* (loin) and *infraspinatus* muscles were found to have no difference in the pH or lower pH in infused carcasses compared to the control, respectively (Farouk & Price, 1994; Fowler et al., 2017).

The changes in pH during storage are important, as higher pH facilitates microbial growth while reduction in pH is associated with growth of lactic acid (producing) bacteria. However, throughout storage, Rinse&Chill carcasses indeed demonstrated to have a lower pH as measured in the loin and this tendency was mostly evident at the end of the shelf- life tested in this study. Considering that lower pH is favoured for prevention of microbial growth, this was expected to affect the microbiological growth, as it will be discussed later in this section. The lack of effect of Rinse&Chill on the pH of the topside throughout storage, can be related to the tendency of the infused solution to be less retained in leg muscles than in the loin (Farouk & Price, 1994). Our results for the initial period of storage agree with the study of bison *longissimus lumborum* and *triceps brachii* muscles which did not find differences in the pH of the muscles due to Rinse&Chill treatment over storage of 7 days (Mickelson & Claus, 2016). In addition, lower pH of meat from Rinse&Chill cull cows was also found during 7 days storage (Kethavath, 2019).

Maintaining a low level of lipid oxidation during storage is very important for the shelf life of lamb, to prevent the accumulation of volatiles that result in unacceptable meat quality. While studies in beef have established threshold TBARS value for sensory acceptability (Campo et al., 2006; Zhang et al., 2019), the actual measured value is species specific and method dependent (Ulu, 2004) and therefore it is difficult to define which lipid oxidation level would be unacceptable to the consumers in this study without a sensory analysis of the samples. Overall, low levels of lipid oxidation and thus TBARS MDA values lower than 1mg/kg meat are expected with vacuum packaging of lamb as the levels of oxidation can be reduced by half or two thirds compared to other packaging systems (like MAP)(Frank et al., 2017; Li, 2019) due to the restricted access to oxygen. The same extent of lipid oxidation in the loin muscles of Rinse&Chill and control carcasses agrees with the results of Li (2019) and Fowler et al.(2017) that did not find differences due to Rinse&Chill treatment in lamb loins stored for 7 days. The greater lipid oxidation in the topside compared to loin muscle, could be

related to differences in the presence of unsaturated fatty acids between the muscles (Popova, 2007) and the higher amount of heme, observed in our study (Figure 7) that has ability to promote lipid oxidation (Bellés et al., 2017). In this line, Popova (2007) found that *semimembranosus* muscle underwent greater lipid oxidation during frozen storage of 240 days compared to the loin in lambs fed with high concentrate diet. It is unclear why there is greater lipid oxidation in Rinse&Chill topsides compared to the control, but looking at the storage times, the greater lipid oxidation in Rinse&Chill is inconsistently limited to two time points, while at most storage periods Rinse and Chill muscles have the same extent of lipid oxidation as the control.

Next to the main role of the heme protein (myoglobin) on meat colour, it also influences lipid oxidation (Faustman et al., 2010). The content of the heme protein has a nutritional value and may play a role in flavour development of meat during cooking (Lombardi-Boccia et al., 2002). The absence of difference in heme protein content in this study between Rinse&Chill and the control throughout extended storage is different with our previous findings of lower heme in Rinse&Chill lamb loins during short storage of 7 days after freezing (Li, 2019), and with the study of Bekhit et al. (2005) that related the lower heme content to a dilution effect of infusing solutions similar to Rinse&Chill ($CaCl_2$, $ZnCl_2$ and water). However, our results align with the study of Erazo- Castrejón et al. (2019) that did not find difference in the heme content between Rinse&Chill and control treatment of pork, immediately after slaughter. The absence of difference in the heme content between Rinse&Chill and the control, points to similar nutritional value between treatments in the context of dietary iron. Overall, the heme content was greater in the topside than in the loin, which is expected as the *semimembranosus* muscle is expected to have higher myoglobin content.

In relation to the state of the heme molecule- the redox state of the myoglobin, consumer prefer a bright red colour associated with oxymyoglobin. The absence of difference between Rinse&Chill and the control in the proportion of oxymyoglobin and metmyoglobin, and even the reduced deoxymoglobin in Rinse&Chill topside compared to the control indicated that Rinse&Chill would lead to equal or even improved colour in some muscles (topside). Previous studies often found significant differences in only one or two of the redox forms during storage of up to 7 days: greater deoxymyoglobin and lower metmyoglobin in Rinse&Chill pork shoulders (Kethavath, 2019); less oxymyoglobin in Rinse&Chill pork loin (Kethavath, 2019); greater oxymyoglobin (day 1, 4 and 7) and deoxymyoglobin (day 7 only) in Rinse&Chill beef with storage for 7 days (Kethavath, 2019); higher deoxymyoglobin in Rinse&Chill in bison meat stored for 1 or 4 days (Mickelson et al., 2018). While our results do not fully resemble the findings in beef, pork and bison meat, they are in agreement to our previous studies in lamb loins treated with Rinse&Chill where no differences in the redox forms of myoglobin were found (Li, 2019).

Lighter and redder meat is preferred by consumers, as meat colour is essential in consumer purchasing decision. Lighter and yellower colour have also been previously reported for lamb carcasses infused with

tenderizing solutions (Farouk & Price, 1994), as well as for Rinse&Chill technology specifically during vacuum display of 7 days (Fowler et al., 2017). However, the present study shows for a first time that this effect is sustained throughout extended storage. Fowler et al. (2017) explained the increased lightness of Rinse&Chill loins by the greater moisture levels of Rinse&Chill carcasses, which in the present study aligns with the findings of lower purge loss (greater moisture) of the Rinse&Chill muscles (Figure 16). The lightness and the redness of the muscles in both treatments were above the defined thresholds for consumer acceptability (9.5 and 34), with the redness in Rinse&Chill muscles being maintained at 95 % probability of being accepted by consumers for up to 105 days (>14.5) (Khliji et al., 2010), compared to 70 days for the control treatment. Our findings of similar redness in the loin of Rinse&Chill and control loins were in agreement with the study of Fowler et al. (2017), while the increased redness of the topside in Rinse&Chill treated carcasses is alike the increased redness found in Rinse&Chill pork shoulders (Kethavath, 2019), Rinse&Chill cow muscles during 7 days of display (Kethavath, 2019), Rinse&Chill bison meat on day 4 of storage (no difference at days 1 and 7), and four Rinse&Chill bovine muscles during 12 days of storage (Bartholomew). Da Cunha Moreira (2018) also reported greater redness, yellowness and lightness on different days of storage (up to 7 days) in bovine muscles from carcasses treated with Rinse&Chill. The greater hue of the Rinse&Chill loins compared to the control and at first and last days of storage, as well as the greater chroma of the topsides during medium duration of storage indicated a better colour intensity of the Rinse&Chill muscles compared to the control.

Our findings of lower purge loss in Rinse&Chill lamb muscles during 4 days of storage disagrees to previous studies on the effect of Rinse&Chill on water- holding capacity in raw meat, that did not find difference in the purge loss of lamb loins stored for 7 days (Fowler et al., 2017; Li, 2019), and pork loin and shoulder stored for 7 days (Kethavath, 2019). The improved water- holding capacity by the Rinse&Chill technology can be assigned to the improved retention of water by myofibrillar proteins in the presence of the rinsing solution containing phosphates (Offer et al., 1989).

The microbiological quality of the carcasses on day 0 (~2 log cfu/g) was comparable to the normally reported values for ovine carcasses (1.5 log cfu/g) (AMPC, 2007). The microbiological quality of the muscles overall was improved by Rinse&Chill treatment, which agreed with the findings of da Cunha Moreira et al. (2018) in cow *quadriceps femoris*, *longissimus* and *triceps brachii* which quantified the Aerobic Plate Count after 7 days of storage. The lower SPC of Rinse&Chill lamb also coincided with the lower pH we found during storage (Figure 5b), which is expected. It is known that VPC lamb is predominately populated by LAB, being facultatively anaerobic, as aerobic microorganisms cannot grow in the absence of oxygen. Keirmeier et al. (2013) showed that the predominant microorganism in VPC lamb was *Carnobacterium* spp.. While a 6 log cfu/g is an overall threshold for detectible spoilage in meat (Gram, 2002; Balasubramanian, 2009), detectible spoilage caused by different groups of microorganisms can vary in the threshold of detection. Namely, lactic acid bacteria and some species of *Enterobacteriaceae* need to reach 8 log cfu/g to result in detectible spoilage,

while thresholds for *Clostridium* spp. are dependent on the control of temperature (Mills et al., 2014). While there are no official microbiological process hygiene criteria for lamb meat in the Food Standards Code, levels of 5-6 log cfu/g can be considered of marginal quality (MLA, 2007) although they will not necessarily result in visible signs of spoilage. Lamb with microbial counts higher than 6 log cfu/g might be considered of unsatisfactory quality, but it might still be organoleptically acceptable for consumers as shown in beef (Small et al., 2012). The lamb in the present study had a better microbiological quality compared to beef stored for the same period of 140 days (AMPC, 2007). A strict hygiene and temperature control should be maintained at all steps of production to avoid marginal quality in lamb meat during extended storage. The models shown in this study can be extended by adding temperature, pH and water activity as parameters for prediction of shelf- life. A comparable presence of *Enterobacteriaceae* in Rinse&Chill and control carcasses, as in our study, was also found in bovine muscles stored for 7 days (da Cunha Moreira et al., 2018; da Cunha Moreira et al.). It is worth noting that the 140-day samples had a high incidence of sour/off odour. As this was not part of the experimental design, quantitative data were not collected. This may be related to microorganisms that cause sensory changes in smaller numbers. This aspect needs further exploration, including sensory acceptability (Frank et al., 2019). It is also noted that the lamb samples used for this study are half individual muscles, i.e. *longissimus* and *biceps femoris* (refer to the method section). The shelf life and quality of meat is affected by surface area/volume ratio. Thus, the shelf life and quality of whole retail cuts, which have a greater surface area/volume ratio than half a muscle, may be different to those of the samples in this study.

Conclusion and recommendations

This study showed that extended shelf life with VPC storage of muscles from lamb carcasses treated both with Rinse&Chill technology and conventional slaughter process is achievable in terms of maintaining acceptable lipid oxidation levels and satisfactory microbiological quality. Rinse&Chill technology resulted in lower pH during storage, increased colour related parameters indicating a better maintained colour during shelf life, lower deoxymyoglobin (topside) and better microbiological quality measured both as lower total levels of bacteria, slower or delayed growth, and lower number of marginal samples. Muscles from Rinse&Chill treated carcasses did not differ to muscles from control in the total heme content, oxymyoglobin and metmyoglobin content. Lamb loins and topsides from Rinse&Chill carcasses also had a better water-holding capacity and therefore yield up to 105 days of shelf- life.

Based on these results, the recommendations are:

- Rinse&Chill® technology is to be applied to lamb carcasses to improve colour and microbiological quality up to 140 days and yield up to 105 days.
- Although laboratory measurements indicate acceptable shelf life of lamb in extended storage, a sensory study would be needed to determine the eating quality of lamb subjected to extended storage and to identify whether there will be differences in eating quality between Rinse&Chill and control treatments.
- It was noted by the researchers that the 140-day samples had a high incidence of sour/off odour. Such sensory study would confirm odour acceptability of lamb in extended storage.

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Supplementary data

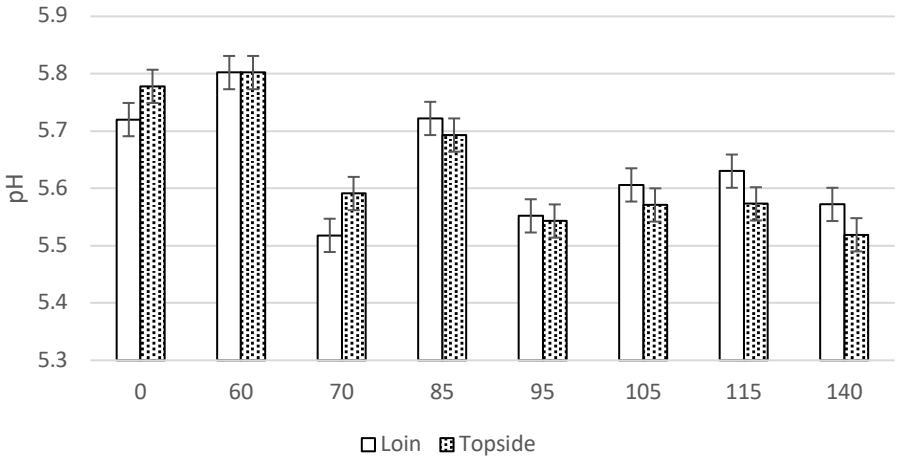

Figure S1. pH of lamb loin and topside muscles with storage (0-140 days), irrespective of treatment. p=0.008.

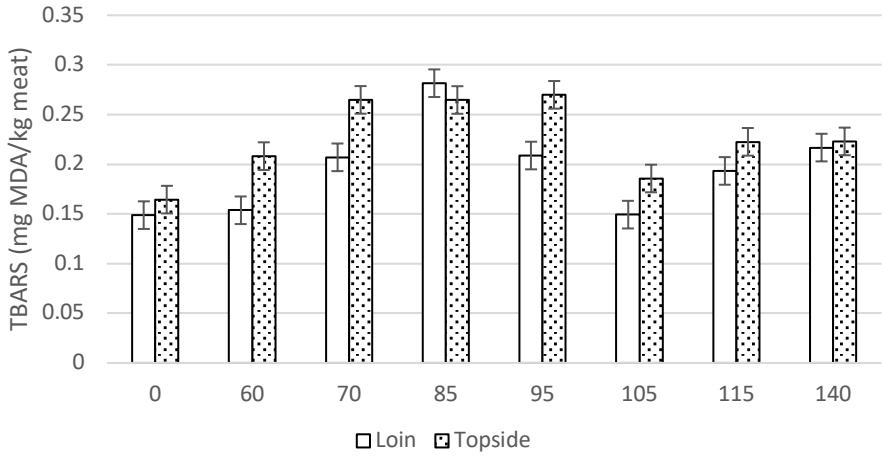

Figure S2. TBARS of lamb loin and topside muscles with storage (0-140 days), irrespective of treatment. p<0.001.

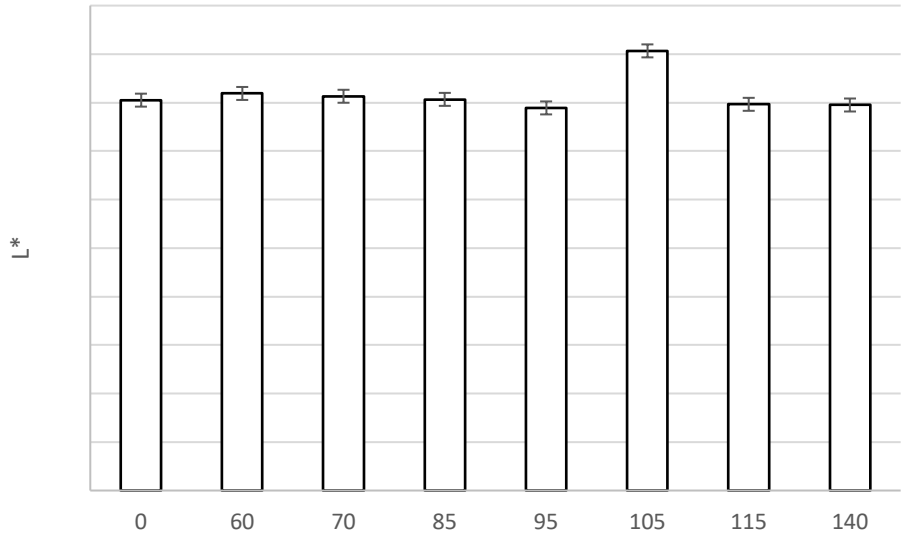

Figure S3. Lightness (L*) of lamb muscles (loin, topside) with storage (0-140 days), irrespective of treatment and muscle type. p<0.001

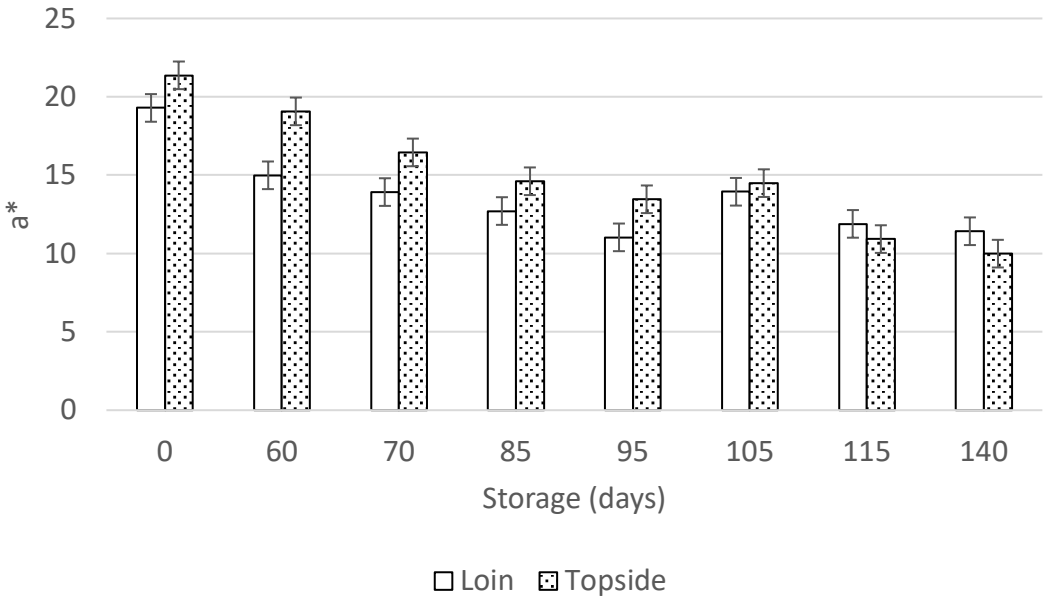

Figure S4. Redness (a*) of lamb loin and topside muscles with storage (0-140 days), irrespective of treatment. p<0.001

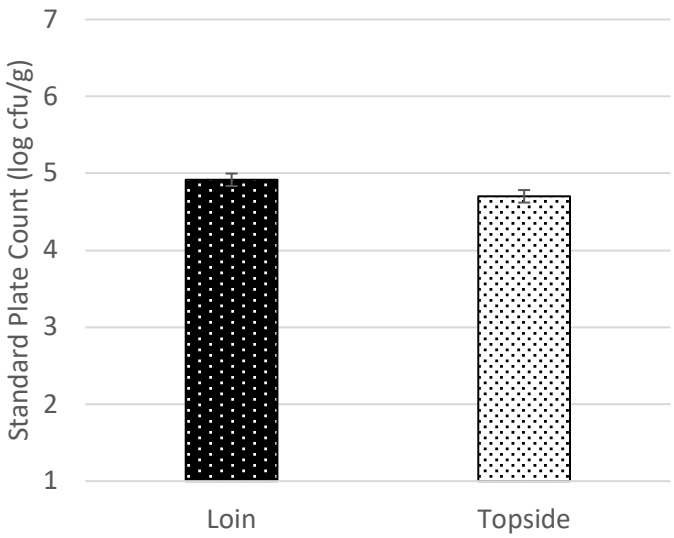

Figure S5. Standard Plate Count (SPC) of lamb loin and topside muscles, irrespective of storage time and treatment.p=0.006

minh.ha@unimelb.edu.au