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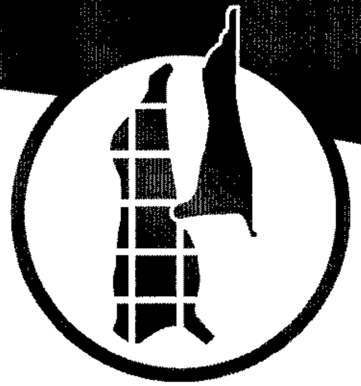

Safe beef carcase – export chilling procedures MSQS.006A

1996

Prepared By:
Australian Meat Technology

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Abstract

The 1993-94 MRC baseline study of the microbiological quality of Australian meat (CS196) identified problems with weekend chilling which were attributed to elevated holding temperatures. MRC funded a project to identify safe beef carcase chilling procedures. As part of that project, temperature patterns have been investigated for chilling and holding beef sides in export abattoirs over weekends. A pattern in which the air temperature was held at 8°C for much of the weekend, then raised to 9.5°C about 15 hours prior to boning, is recommended. Deep butt, carcase surface and air temperatures were monitored throughout the weekend trials. Water activity (a measure of surface dryness) and microbiological tests were done at intervals for each trial. Water activity fell during the first hours of chilling, then gradually increased to plateau after about 30 hours. The drying would have inhibited microbiological growth, particularly on a test site over the cube roll. *E. coli* was rarely detected on the test sides and other coliforms were present too infrequently for general conclusions to be drawn about the chilling regimes that were investigated. Use was therefore made of computerised predictive models for growth of *E. coli* and a test organism was used for the purpose of validating predictions. When the recommended chilling pattern was used, predicted and actual increases in numbers were generally 10% or less of those when using the pattern currently in routine use for weekend chills in one of the trial abattoirs. Inclusion of a final three-hour re-warming phase in the 18-hour conditioning phase had little impact on bacterial numbers while seeming to obviate hard fat problems.

Summary Report

Most export plants use chilling cycles that broadly consist of a loading phase, chilling phase and a holding phase. The control of beef chillers in most export meat plants is such that different temperatures and fan speeds can be set for different stages of the overnight and weekend chilling cycles. CSIRO has recommended the use of a chilling phase with an air-on evaporator coil temperature close to 0°C followed by a holding phase that maintains the carcase surface temperature no higher than 8°C.

The MRC baseline study of the microbiological quality of Australian meat, CS196, found problems with weekend chilling which were attributed to elevated holding temperatures. From the study, CSIRO recommended that the holding temperatures over weekends be no higher than 7°C. A meat temperature of 7°C is too cold for many boning rooms because the hard fat which results gives rise to industrial problems, especially with grain-fed cattle. Therefore many plants continue to hold carcases at 10°C - the highest temperature allowable under the Export Meat Orders.

Holding temperatures up to the allowable temperature of 10°C do not lead to excessive bacterial growth during overnight chilling due to the short time periods involved. However during weekends, particularly long weekends, the holding period at 10°C can exceed 60 hours. The main focus of this module of the project has therefore been to evaluate industrially acceptable chilling cycles that adequately control growth of pathogenic organisms during weekends.

For many of the pathogens, the temperature region 7-10°C is approaching the minimum temperature for growth and even a slight drop in temperature has a very significant effect on their growth rate and on whether or not growth even occurs.

In the project, the effect of closely controlling temperature and surface dryness (measured as water activity) to minimise or prevent growth of *E. coli* and similar pathogens during weekend chilling was investigated. In addition to in-plant investigations, use was made of available predictive models for growth of bacteria to predict *E. coli* growth under various conditions of temperature and water activity. To illustrate, using a model developed by MIRINZ, if for a 48 hour period during the weekend the temperature is reduced by 2°C from 10°C to 8°C, then there would be almost an 8-fold or almost 1 log unit difference in numbers.

Trials were conducted during weekend chills on 15 occasions. In each trial, six sides from those loaded into the test chiller were selected for assessment. Trials were undertaken in three plants; most were in one chiller at Plant A. The test sides were in the weight range 120 to 190 kg. The chilling regimes were selected in consultation with abattoir management. The air temperature regimes were selected in order to give satisfactory chilling rates and acceptably soft and boneable fat at the end of the chilling cycle.

Several chilling patterns were investigated during the trials. Four initial trials were carried out using plant A's current chiller operating pattern for weekends to establish the base conditions. Samples were collected from sides for microbiological assessment and measurement of water activity during Trials 3 and 4. During Trial 5, a conditioning phase was introduced during which the air was warmed for three hours prior to boning by operating the plant in reverse cycle mode.

Trial 6 was used to monitor conditions and bacterial growth over a long (three-day) weekend and for trials 7 and 9, a lower holding temperature was utilised. Weekend conditions in two other export plants were monitored in Trials 8 and 10. During Trials 11 to 15, an approved test organism, *Klebsiella oxytoca,* was applied to sites on the test sides and the chiller was

operated under conditions designed to control the growth and produce carcases with a fat hardness acceptable for boning.

Temperatures of the deep butt, surface butt, surface cube roll, surface brisket and air over the butt and brisket of each of the test sides were logged at 15 minute intervals. An air velocity meter was used to measure air speeds adjacent to the test sides. Water activity of subcutaneous fat tissue from the cube roll and brisket sites was measured using a water activity meter.

Surface tissue samples were collected from the sides at the start of chilling, after overnight chilling and the end of each chilling cycle. The tissue excision samples from each side were tested separately for total viable count, coliforms, and *E. coli*.

Temperature monitoring of the current weekend chilling pattern at Plant A indicated that the system is capable of providing very precise control of temperature. The air temperature over the sides and therefore the surface temperature during the weekend holding period was typically in the range 9°C to 10°C.

As stated earlier a reduction in temperature of only 1°C to 2°C can have a large effect on growth rate of *E. coli* at temperatures below 10°C. The chilling pattern utilised at the plant was modified by lowering the holding temperature to 8°C to 8.5°C up until the final 10 to 15 hours of the holding period when the air temperature was allowed to rise to the abattoir's normal holding temperature of 9.5°C to reduce the risk of industrial problems due to hard fat. A reverse-cycle warming phase was also included for the final three hours to evaluate its effect on bacterial growth.

There were consistently significant falls in water activity during the first hours of chilling, followed by a gradual increase and a levelling after 20 to 30 hours. There were clear differences in the measured water activity values between the brisket and cube roll surface tissue, the brisket having a consistently higher value.

The total viable count and counts of coliforms and *E. coli* from the first series of trials reflected the initial normal contamination on the sides from slaughter and dressing and the increases in numbers of those organisms. *E. coli* was rarely detected and other coliforms were detected too infrequently to make it possible to draw any real conclusions about the effect of chilling practice on proliferation of the organisms except for the two trials which occurred over long weekends where there were definite increases in numbers.

In later trials small numbers of the test organism *Klebsiella oxytoca* were applied to designated test areas. After overnight chilling, counts of the organism were generally less than at the start of chilling, suggesting that it suffered shock or injury. By the end of the weekend chill numbers had increased on the brisket by approximately 100-fold (2 log units) and on the cube roll by over 30-fold (1.5 log units). In most cases the rewarming phase, which completed the patterns of some trials, resulted in little further increase. It never resulted in more than a three-fold increase. The benefit of employing a chilling pattern with a holding phase lower than currently being used at Plant A was clearly demonstrated.

Data were obtained using predictions from models for *E. coli* O157:H7 (USDA model), other strains of *E. coli* (CSIRO, MIRINZ) and *K. oxytoca*. The conditions utilised for the predictions were those of Trials 11, 12 and 15. Trial 11 was carried out using the weekend chilling program currently used by Plant A. The set-point temperatures for Phase 3 (the holding phase of the cycle) for Trials 12 and 15 were, respectively, 1.0°C and 1.5°C lower than for Trial 11.

The beneficial effect of reducing the set-point temperature for the holding phase by 1.5°C was clearly indicated by the prediction models. For the chilling program of Trial 15, the predicted increases in numbers of *E. coli* and of the test organism *K. oxytoca* were generally only 10% or less of those for the program currently in routine use for weekend chills. The

actual test results for *K. oxytoca* from samples tested during the trials were consistent with the predictions.

For the final, rewarming, phase a decision was made to allow the surface temperature to rise to not more than 20°C. In the event, on no occasion did the temperature rise to 18°C. The re-warming pattern was sufficient to avoid problems due to hard fat. After weekend chilling under the current regime, plant management has often found it necessary to subject some carcases to a physical fat softening procedure. The sides subjected to the 'hot gas' conditioning during the trials did not require this treatment.

From the prediction equations it has also been calculated that even if surface temperatures had reached 20°C and the period of conditioning had been as long as five hours, the expected increases would not have exceeded three-fold (0.5 log units).

It is concluded that it is possible to use a weekend chilling procedure which prevents hard fat conditions while avoiding holding the beef sides for extended periods at a temperature at which significant growth of *E. coli* or other pathogens can occur.

Recommended procedures for loading, chilling, holding and conditioning (equilibration and rewarming) phases are as follows:

Phase	Fan Speed	Temperature (°C)	Time (h)
Load	50% - 60%	10°C - 12°C	~2
Chill	100% (1.0 - 1.2m/s)	0°C - 5°C	8 - 12
Hold	40% - 50% (0.3 - 0.5 m/s)	7°C - 8°C	45 - 50
Conditioning	40% - 50% (0.3 - 0.5 m/s)	10°C	10 - 15
Rewarming (optional)	100%	15°C - 20°C	3

Main Report

Background

The MRC baseline study of the microbiological quality of Australian meat, CS196, found problems with weekend chilling. These were attributed, mainly, to elevated holding temperatures. From the study, CSIRO recommended that the holding temperatures over weekends be no higher than 7°C.

Although 7°C is tolerated in some boning rooms, it is too cold for many because the hard fat produced gives rise to industrial problems especially with grain-fed cattle. Therefore many plants hold carcases at 10°C, the highest temperature allowable under the Export Meat Orders.

For many of the pathogens, the temperature region 7-10°C is approaching the minimum temperature for growth and even a slight drop in temperature has a very significant effect on their growth rate and on whether or not growth even occurs.

In addition, the surface drying that occurs during chilling means that moisture is less freely available to bacteria. This reduced moisture availability is also inhibitory to bacteria.

The control of beef chillers in most export meat plants is such that different temperatures and fan speeds can be set for different stages of the overnight and weekend chilling cycles. Chilling cycles typically consist of a loading phase, chilling phase and a holding phase.

Most plants broadly follow recommendations contained in the CSIRO handbook 'Production of Chilled Meat for Export'. These suggested a chilling phase with an air-on evaporator coil temperature close to 0°C followed by a holding phase that maintains the carcase surface temperature no higher than 8°C. However both the chilling temperature and holding temperature are often compromised to an extent in order to produce carcases with fat hardness that does not lead to industrial dispute or workers compensation or safety problems during boning.

Holding temperatures up to the allowable temperature of 10°C do not lead to excessive bacterial growth during overnight chilling due to the short time periods involved. However during weekends, particularly long weekends, the holding period at 10°C can exceed 60 hours. The main focus of this project has therefore been to evaluate industrially acceptable chilling cycles that adequately control growth of pathogenic organisms.

Investigations centred on the effect of closely controlling temperature and surface dryness (measured as water activity, a_w which numerically is directly related to the relative humidity of the surrounding air:- $a_w = RH/100$) to minimise or prevent growth of *E. coli* and similar pathogens.

Predictive modelling

Several groups of researchers have developed and modified models to describe the growth behaviour of selected bacteria on meats and other foods.

Predictions of *E. coli* growth under various conditions of temperature, water activity, pH and available oxygen have been made using computer modelling programs such as the MIRINZ Food Product Modeller and the USDA Pathogen Modelling Program, PMPWIN version 5.1.

Effect of temperature on the amount of growth

To illustrate the effect of holding temperature, predicted increases in numbers of *E. coli* at temperatures relevant to carcase chillers have been calculated using the MIRINZ program and are presented below for a water activity of 0.995.

Temperature (°C)	Increase in 48 h
10	50-fold
9	17-fold
8	6.9-fold

Therefore, if for the 48 hour period the temperature is reduced by 2°C from 10°C to 8°C, then there would be almost an 8-fold difference in numbers. That is, for two chillers, one held at 10°C, the other at 8°C, after the 48 hour period there would be an 8-fold , or almost 1 log unit difference in numbers.

Effect of water activity on the amount of growth

The relationship between water and meat spoilage organisms was studied extensively by Scott who coined the term water activity in this context. The importance of surface drying in restricting microbial growth on beef carcases was demonstrated by Scott and Vickery (1939)*. Decreasing a_w results in progressively decreasing rates of growth of bacteria and other microorganisms. Initially the water activity of moist, warm carcases exceeds 0.99. The water activity falls during the early phase of chilling as the surface dries but as chilling progresses the a_w rises again. A typical general pattern is shown in Figure 3.

Growth at 9°C in 48 h (values from USDA program):

Water activity	Increase in 48 h
0.99	22.6-fold
0.98	14.9-fold

Therefore for two chillers or different points on the carcase surface held at 9°C, one with water activity at 0.99 the other at 0.98, after the 48 hour period there would be a 0.4 log difference in numbers.

Effect of temperature on lag time

In addition to its influence on the rate of increase in numbers of bacteria, the temperature influences the lag time or period between when the bacteria arrive on the surface of freshly dressed bodies and when they actively begin to increase in numbers. This can be demonstrated for *E. coli* O157:H7 from the USDA program. A water activity of 0.986 has been selected for this purpose:

* Scott, WJ & Vickery, JR (1939) CSIR Bull. No.129

Temperature (°C)	Lag time (h)
30	2.4
25	3.8
20	7.6
15	18.7
12	47.3
11	59.1
10	74.5
9	94.7
8	N.G.
7	N.G.

N.G. No growth

Effect of water activity on lag time

In addition to its effect on the rates of growth of bacteria, changes in water activity are known to influence lag time. From the USDA program, and using a temperature of 9°C the effect of drying the carcase surface to reduce the a_w can be seen to increase the likely lag time for *E. coli* O157:H7.

Water activity	Lag time (h)
0.990	83.9
0.988	88.8
0.986	94.7
0.984	101.5
0.982	109.6
0.980	122.5

Methodology

The majority of the chilling trials were carried out within the one plant. The same chiller was used on all occasions except Trials 1 and 15 and held approximately 80 bodies on four rails. The chilling regimes were selected in consultation with abattoir management. The air temperature regimes were selected in order to give satisfactory chilling rates and acceptably soft and boneable fat at the end of the chilling cycle.

Trials were conducted during weekend chills on 13 occasions. In addition two trials (Trials 14 and 15) from Project TR.006 are reported. In each trial, six sides from those loaded into the test chiller were selected for assessment. The test sides, usually from steers, were in the weight range 120 - 190 kg and were located in approximately the same positions in the chiller for each trial. Three sides were on outside rails and the other three on inside rails so that they were surrounded by other sides.

The chiller which uses an ammonia recirculation system was controlled by means of back-pressure control which has the ability to provide a constant temperature over extended holding periods. The selected air temperature was set by entering the required evaporator air-on set-point temperature into the computer controlling the system.

Chill cycles

The regimes used for weekend chills are summarised below.

	Fan speed (% rated capacity)	Air-on Setpoint Temperature (°C)	Duration (h)	Comments*
Trial 1				
First phase	100	8.5	8	Current
Second phase	60	9.5	12	pattern
Third phase	40	9.5	100[†]	
Trial 2				
First phase	100	8.5	8	Current
Second phase	60	9.5	12	pattern
Third phase	40	9.5	100[†]	
Trial 3				
First phase	100	8.5	8	Current
Second phase	60	9.5	12	pattern - micro
Third phase	40	9.5	100[†]	sampling
Trial 4				
First phase	100	8.5	8	Current
Second phase	60	9.5	12	pattern - micro
Third phase	40	9.5	100[†]	sampling
Trial 5				
First phase	100	8.5	8	Current
Second phase	60	9.5	12	pattern
Third phase	40	9.5	100[†]	plus conditioning
Conditioning	100	18.0**	3	
Trial 6				
First phase	100	8.5	8	
Second phase	60	8.5	4	Long
Third phase	40	9.5	100[†]	weekend
Trial 7				
First phase	100	8.5	8	Modified
Second phase	60	8.5	37	pattern
Third phase	40	9.5	100[†]	
Trial 8				
First phase	100	3.0	10	
Second phase	60	7.0	10	Plant B
Third phase	40	7.0	10	
Fourth phase	20	7.0	100[†]	

* Plant A unless specified
** Maximum air temperature recorded
† Phase manually terminated

Fan speed (% rated capacity) | Air-on Setpoint Temperature (°C) | Duration (h) | Comments*

Trial 9				
First phase	100	8.5	8	
Second phase	60	8.5	12	Modified pattern
Third phase	40	8.5	25	
Fourth phase	40	9.5	7	
Fifth phase	40	12.0	4	
Trial 10				
First phase	50% on	4.0	6	Plant C
Second phase	50% on	10.0	100†	Long weekend
Trial 11				
First phase	100	8.5	12	
Second phase	60	8.5	8	Test organism applied
Third phase	40	9.5	100†	
Conditioning	100	18.0**	3	
Trial 12				
First phase	100	8.5	12	
Second phase	60	8.5	8	Test organism applied
Third phase	40	8.5	100†	
Conditioning	100	20.0**	3	
Trial 13				
First phase	100	8.0	12	
Second phase	60	8.0	37	Test organism applied
Third phase	40	9.5	100†	
Conditioning	100	18.0**	3	
Trial 14				
First phase	100	8.0	12	
Second phase	60	8.0	37	Test organism applied
Third phase	40	9.5	100†	
Conditioning	100	20.0**	3	
Trial 15				
First phase	100	8.0	12	
Second phase	60	8.0	37	Test organism applied
Third phase	40	9.5	100†	
Conditioning	100	15.0**	3	

* Plant A unless specified
** Maximum temperature recorded
† Phase manually terminated

Four initial trials were carried out using the plant's current chiller operating pattern for weekends to establish the base conditions. Samples were collected from sides for microbiological assessment and measurement of water activity during Trials 3 and 4. During Trial 5, a conditioning phase was introduced during which the air was warmed for three hours prior to boning, by operating the plant in reverse cycle mode.

Trial 6 was used to monitor conditions and bacterial growth over a long (three-day) weekend and for Trials 7 and 9, a lower holding temperature was utilised. Weekend conditions in two other export plants were monitored in Trials 8 and 10. During Trials 11 to 15, sites on the sides were inoculated with a test organism and the chiller operated under conditions designed to control the growth and produce carcases with a fat hardness acceptable for boning. Programming problems during Trials 13 and 14 resulted in the temperatures failing to follow the intended pattern.

Temperature measurement

Thermocouples were used to measure temperatures of the deep butt, surface butt, surface cube roll, surface brisket and air over the butt and brisket of each of the 6 test sides in each chiller during each trial. Surface temperatures were measured by inserting the sensing tip of the thermocouples less than a millimetre under the surface adjacent to the sites sampled for microbiological analysis and water activity measurement. Temperatures were logged on calibrated data loggers at 15 minute intervals.

Air speed measurement

A TSI Velocicalc Model 8346 air velocity meter was used to measure air speeds adjacent to the thermocouples measuring surface butt, surface cube roll and surface brisket temperatures.

Water activity measurement

The water activity was measured by excising samples of the subcutaneous fat tissue from the cube roll and brisket sites. Each sample was 38 mm diameter and approximately 1 mm deep to suit the water activity meter cup. The water activity was measured using an 'Aqualab' Model CX-2 Water Activity Meter.

Microbiological sampling and testing

Trials 3 - 10

Surface tissue samples (≈6.5 cm^2) were excised from the cube roll and brisket of each test side. The samples were collected while the sides were in the chillers at the start, after overnight chilling and the end of each chilling cycle. The tissue excision samples from each side were tested separately.

Microbiological enumeration
Samples that were excised from the test sides before and after chilling as described above were held under refrigeration at 4°C until required for testing. Testing was undertaken as soon as practicable after the samples had been collected and on all occasions was completed within two hours of sampling.

The samples were tested as follows:

- 25 mL volumes of Butterfield's diluent were added to the samples and stomached for one minute.
- Appropriate dilutions of the samples were enumerated on Petrifilm *E. coli*/coliform plates and on Petrifilm aerobic plates.
- The *E. coli*/coliform plates were incubated at 37°C for 24 h, counted, then checked after a further 24 h incubation. The aerobic plates were held at 25°C (samples for Friday, Saturday), or near that temperature (Monday samples) for up to 72 h prior to counting.

Trials 11 - 15

Test organism
Klebsiella oxytoca NRRL B-199, which is approved by AQIS and FSIS as a suitable organism for assessing intervention treatments was used. The growth of this organism at several temperatures between 5 and 37°C has been investigated. The results of that investigation are reported separately (Appendix B)

On the first day of each of the three trials an overnight broth culture of *K. oxytoca* was subcultured and incubated for approximately 2 hours. The turbidity of the culture was measured and the value was used to estimate the number of colony forming units per unit volume. Approximately 20 minutes before the suspension was required for application to beef sides, the culture was diluted to give a suspension that was estimated to have a count of approximately $5x10^3$ bacteria per mL. A portion was transferred to a sterile plastic bag containing a sterile applicator sponge which was in turn placed within a lidded plastic beaker for transfer to the test chillers.

For each trial, six sides were tagged as test sides for application of the suspension. These were located at various positions within the chiller as described earlier. Areas over the cube roll and brisket, each measuring approximately 10 cm x 10 cm, were painted with the suspension using the applicator sponge. The areas were marked for later identification by scoring, using a scalpel blade. After an interval of approximately 10-20 minutes, tissue (area approximately 6.5 cm^2) was excised from each painted site for microbiological testing.

The sides were sampled again on Saturday after they had been chilled overnight and twice on Monday morning - immediately prior to the commencement of conditioning and again shortly before they were required to be moved out of the chiller for boning.

Although the test organism poses no risk to health or shelf life of the product, care was taken to avoid any cross-contamination between test sides and others. The treated areas of test sides were trimmed immediately after the Monday morning sampling had been completed.

Excised samples were tested as described above.

Project results

Temperature regime of chills

Typical temperature records for each of the 15 trials undertaken at three plants are included with this report as Appendix A.

Current pattern

A typical plot of the current weekend chilling pattern at Plant A (Figure 1) shows that the system is capable of providing very precise control of temperature. The air temperature over the sides and therefore the surface temperature during the weekend holding period was typically in the range 9°C to 10°C.

The chilling cycle provided reliable compliance with the Export Meat Orders with regard to both rate of fall of deep butt temperature and the carcase surface temperature for the range of side weights encountered (up to 190 kg) but surface temperatures do not approach the 7°C recommended by CSIRO and specified by ARMCANZ in Australian Standard for Hygienic Production of Meat for Human Consumption.

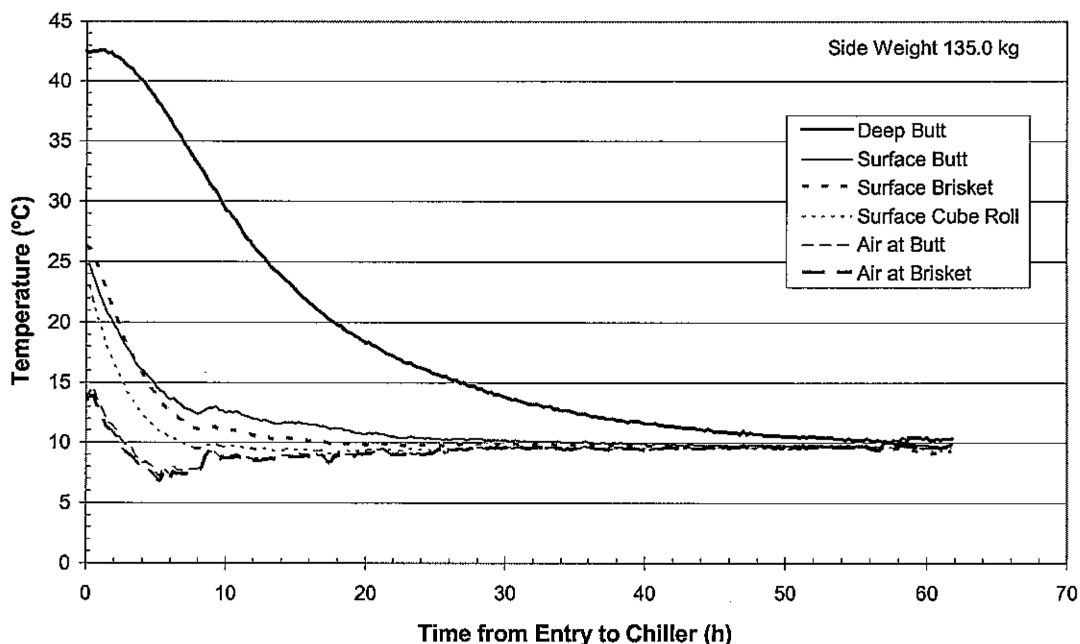


Figure 1: Typical current weekend chilling cycle

Modified pattern

As demonstrated earlier a small reduction in temperature of only 1°C to 2°C can have a large effect on the growth rate of *E. coli* at temperatures below 10°C. Permission was obtained from abattoir management to modify the current chilling pattern utilised at the plant by lowering the holding temperature to 8°C to 8.5°C. During the final 10 to 15 hours of the

holding period, the air temperature was allowed to rise to the abattoir's current normal holding temperature of 9.5°C to reduce the risk of industrial problems due to hard fat. A reverse-cycle warming phase was also included to evaluate its effect on bacterial growth.

A typical plot of this chilling regime is presented in Figure 2. At about 45 h a rise in air and carcase surface temperatures of one degree is evident. During the trials the re-warming phase routinely commenced at 3.00 am and ceased before hot carcases arrived at the chiller complex. The absence of a load for the refrigeration system during the 'hot gassing' operation meant that the rise in temperature during reverse cycling in the test chiller was restricted.

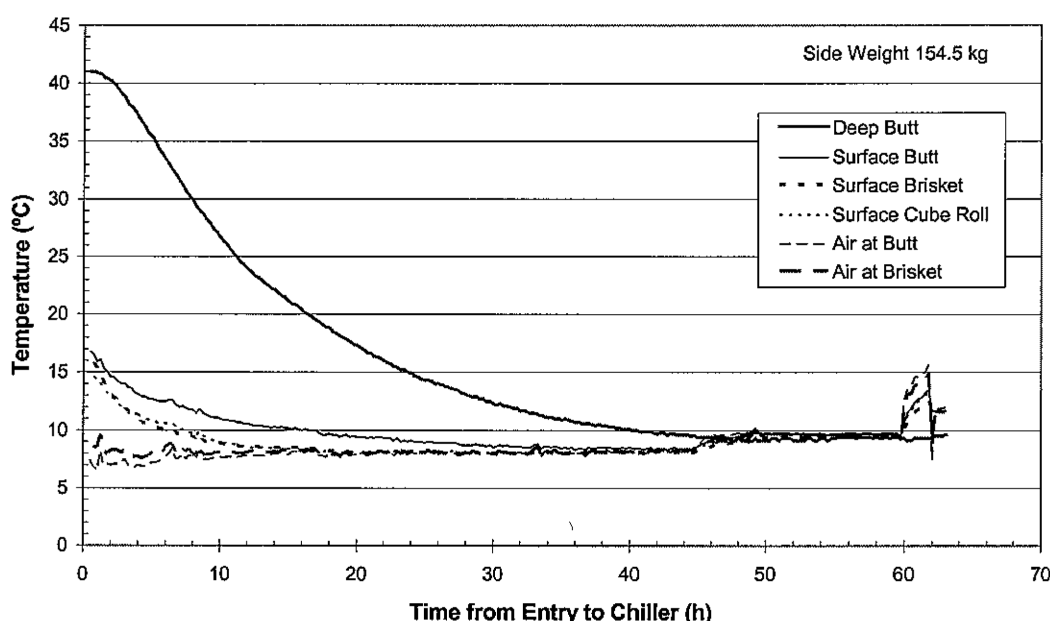


Figure 2: Modified weekend chilling cycle

Water activity profile

The pattern of change of water activity on the surface of the brisket and cube roll during the first four benchmarking trials is presented in Figure 3. Samples were collected at intervals of 3 to 6 hours during the first 20 hours, then after approximately 40 hours, immediately prior to the commencement of re-warming (approx. 60 hours) and where relevant, after re-warming.

There were consistently significant falls in water activity during the first hours of chilling, followed by a gradual increase and a levelling after 20 to 30 hours. The error bars show the variation, expressed as standard deviation, between the four runs. For each of the four runs, both the set-point temperature and the air speed changed after 8 hours, and at 20 hours the air speed reduced again. There were clear differences in the measured water activity values between the brisket and cube roll surface tissue - the brisket having consistently higher a_w and above the level at which growth of *E. coli* would be expected at the holding temperature of 10°C. There was an obvious difference in the physical nature of the thin discs of surface tissue removed between the two sites from around 18 hours after the start of chilling.

At Plant B, although the air temperature was pulled down to below 0°C and held at 6°C to 7°C, the pattern of water activity change was similar to that shown in Figure 3.

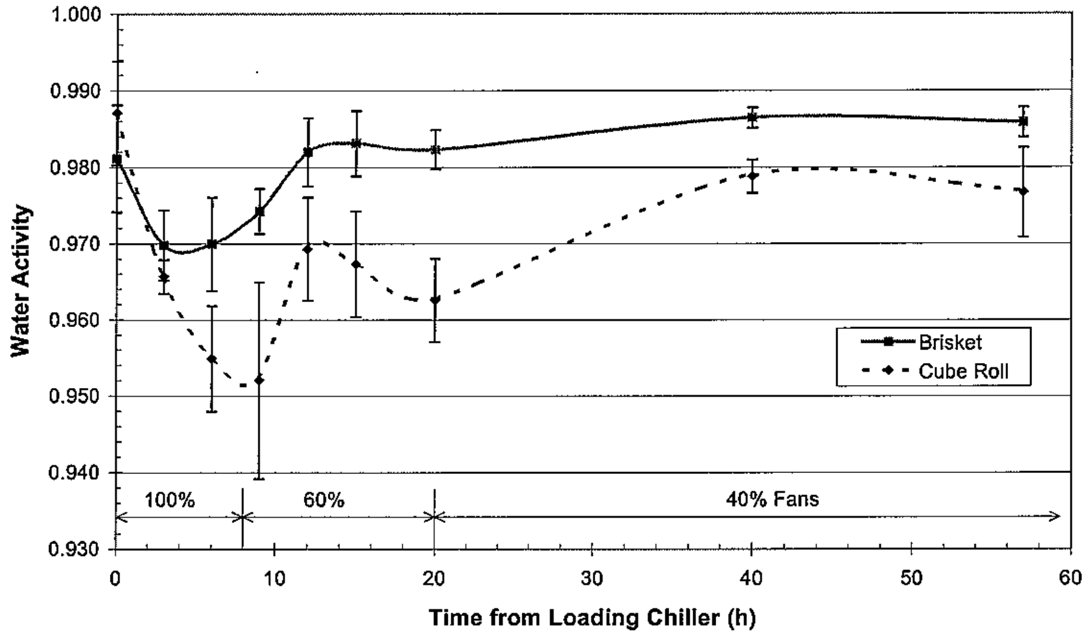


Figure 3: Carcase water activity during weekend chilling (averaged data for all runs using current pattern)

Air velocity

In the test chiller there was a measurable reduction in air velocity when the fan speed was reduced after eight hours chilling. However the further reduction in fan speed at 20 hours did not result in a measurable further reduction in air velocity over the test sides (Table 1). There was a greater influence on the velocity of the actual positions of the sides. The six sides monitored in each run were located in approximately the same positions in the test chiller each time. Sides 1, 2 and 3 were on outside rails and sides 4, 5, and 6 were on inside rails. The air velocity over side 5 was consistently higher than over the other sides. There was not a detectable relationship between measured air velocity and water activity.

Table 1: Air velocity over carcase surfaces as affected by fan speed. Average of four trials in test chiller at Plant A

Fan speed setting(%)	Site		
	Butt	Cube roll	Brisket
100	1.0	0.8	0.9
60	0.6	0.6	0.5
40	0.7	0.5	0.6

Microbiology

After two initial trials, samples from each of the test sides were enumerated for total viable count, coliform organisms and *E. coli* on Petrifilm plates. For the first eight trials these counts reflected the initial normal contamination on the sides from slaughter and dressing and the increases in numbers of those organisms. *E. coli* was rarely detected and other coliforms were detected too infrequently to make it possible to draw any real conclusions about the effect of chilling practice on proliferation of the organisms, although for the two trials which occurred over long weekends there were measurable increases in numbers of coliforms after 80 to 85 hours.

Consequently, from Trial 11 small numbers of the test organism *Klebsiella oxytoca* were applied to designated test areas on the test sides on each of the brisket and cube roll regions. Based on the test results from Trials 11 and 12, where after overnight chilling some sites tested yielded none of the test organism, it was decided to increase the concentration of the bacterial suspension applied (Trials 13, 14, 15). In the early stages of chilling it was apparent that the organism suffered shock. Invariably the counts on Petrifilm *E. coli* plates were lower after overnight chilling than when the suspension was first applied.

The total viable counts and coliform counts for Trials 3 to 15 are presented in Tables 2 and 3 respectively.

Table 2: Total viable counts ($\log_{10}$ per cm^2). Trials 3-10, normal contamination; Trials 11-15, test organism *K. oxytoca* applied.

Trial	Stage of chill: Start (0 h)		Overnight (≈20 h)		After weekend (≈60 h)[1]		After conditioning (≈63 h)		After long weekend (≈80 h)[2]	
	Brisket	Cube roll	Brisket	Cube roll	Brisket	Cube roll	Brisket	Cube roll	Brisket	Cube roll
3[3]	ND	ND	ND	ND	3.2	3.0				
4	1.8	1.7	1.7	2.1	2.5	2.9				
5	1.8	2.3	1.7	2.1	2.1	2.4	2.5	2.7		
6	2.4	2.3	ND	ND	2.5[1]	2.4[1]			3.9	3.4
7	2.0	2.3	ND	ND	2.2	1.8				
8	2.6	2.1	ND	ND	2.8	1.7				
9	1.7	1.7	ND	ND	1.7	2.9				
10	1.8	2.1	1.9	2.3	2.2	2.7			2.9	3.6
Test	organism									
11	2.5	3.0	2.1	2.1	3.7	4.2	3.9	4.0		
12	3.0	2.9	2.7	2.8	3.6	3.2	4.0	3.7		
13	2.7	2.8	2.3	2.1	4.1	4.5	4.6	4.4		
14	2.5	3.2	1.8	2.5	4.4	4.7	5.2	5.2		
15	4.0	3.8	4.6[3]	4.6[3]	5.2	4.9	5.5	4.7		

[1] After 72 h for Trials 6, 10 ND Not determined

[2] After 80 h Trial 6, after 86 h Trial 10

[3] No microbiology done for Trials 1 & 2

[4] For Trial 15, tests done at 42 h rather than 20 h.

Table 3: Coliform counts ($\log_{10}$ per cm^2). Trials 3-10, normal contamination; Trials 11-15, test organism *K. oxytoca* applied.

Trial	Stage of chill: Start (0 h)		Overnight (≈20 h)		After weekend (≈60 h)[1]		After conditioning (≈63 h)		After long weekend (≈80 h)[2]	
	Brisket	Cube roll	Brisket	Cube roll	Brisket	Cube roll	Brisket	Cube roll	Brisket	Cube roll
3[3]	0.2	0.1	ND	ND	1.0	0.3				
4	0	-0.04	-0.2	0.04	0.02	-0.06				
5	0.1	0.7	-0.2	0.2	0.1	0.4	0.2	0.2		
6	0.05	0.2	ND	ND	0.1	0			0.5	0.7
7	0.1	0.1	ND	ND	0.1	0.4				
8	-0.3	-0.3	ND	ND	-0.2	-0.4				
9	0.05	0.1	ND	ND	0.04	0.1	ND			
10	0	0.1	-0.2	-0.1	0.1	0.8			0.1	1.3
Test	organism									
11	1.4	2.2	0.7	0.9	3.3	4.0	3.1	3.4		
12	1.0	1.3	0	0.4	1.9	1.6	2.4	1.7		
13	2.3	2.6	1.3	1.7	3.6	4.1	4.4	4.3		
14	2.4	3.0	1.3	1.9	4.1	4.8	4.9	4.9		
15	3.8	3.7	4.4[4]	4.4[4]	4.9	4.7	5.3	4.7		

Table 4: Actual and predicted increases in numbers of bacteria using chill patterns from specified trials.

Trial	*K. oxytoca* Actual		*K. oxytoca* Predicted		*E. coli* - predicted USDA (0.986)		USDA (0.995)		CSIRO		MIRINZ	
	Brisket	Cube Roll	Brisket	Cube Roll	Brisket	Cube Roll	Brisket	Cube Roll	Brisket	Cube Roll	Brisket	Cube Roll
11	1.87	1.74	3.90	3.49	2.52	2.24	3.70	3.26	3.48	3.00	2.94	2.53
12	1.10	0.30	3.29	3.34	1.97	2.21	2.81	3.14	2.78	2.82	2.69	2.72
15	1.10	1.00	2.70	2.70	0.96	1.00	1.31	1.37	2.10	2.25	1.90	2.00

Table 4 and Figures 4 and 5 (for brisket and cube roll respectively) were produced with data obtained using predictions from models for *E. coli* O157:H7 (USDA model), other strains of *E. coli* (CSIRO, MIRINZ) and *K. oxytoca*. The conditions utilised for the predictions were those of Trials 11, 12 and 15. Trial 11 was carried out using the weekend chilling program currently used by Plant A. The set-point temperatures for Phase 3 (the holding phase of the cycle) for Trials 12 and 15 were, respectively, 1.0°C and 1.5°C lower than for Trial 11. The table presents the actual increases in numbers of *K. oxytoca* measured in Trials 11, 12, and 15 together with predicted values for that organism and *E. coli* using various predictions. The figures show the relative increases in *E. coli* and *K. oxytoca* rather than absolute increases in order to permit easy comparison between the models. The values for each of the modified patterns are expressed as proportions of increases predicted from the current weekend chilling pattern. Also included on the plots are values for *K. oxytoca* calculated from actual increases which occurred.

The beneficial effect of reducing the set-point temperature for the holding phase by 1.5°C is clearly evident in the figures. For the chilling program of Trial 15, the predicted increases in numbers of *E. coli* and of the test organism *K. oxytoca* were generally only 10% or less of those for the program currently in routine use for weekend chills. The actual test results for *K. oxytoca* from samples tested during the trials were consistent with the predictions. It should be noted that none of the predictions of increase in numbers have made allowance for a lag phase which will almost certainly precede the growth phase during the holding period.

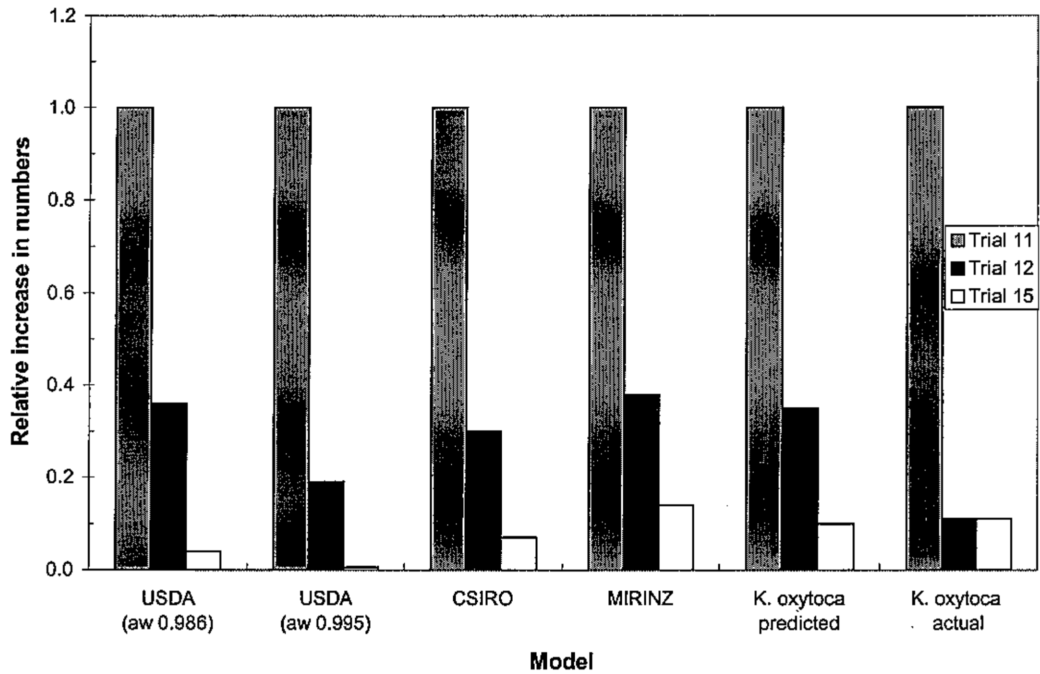


Figure 4: Relative increases during weekend chills - Brisket

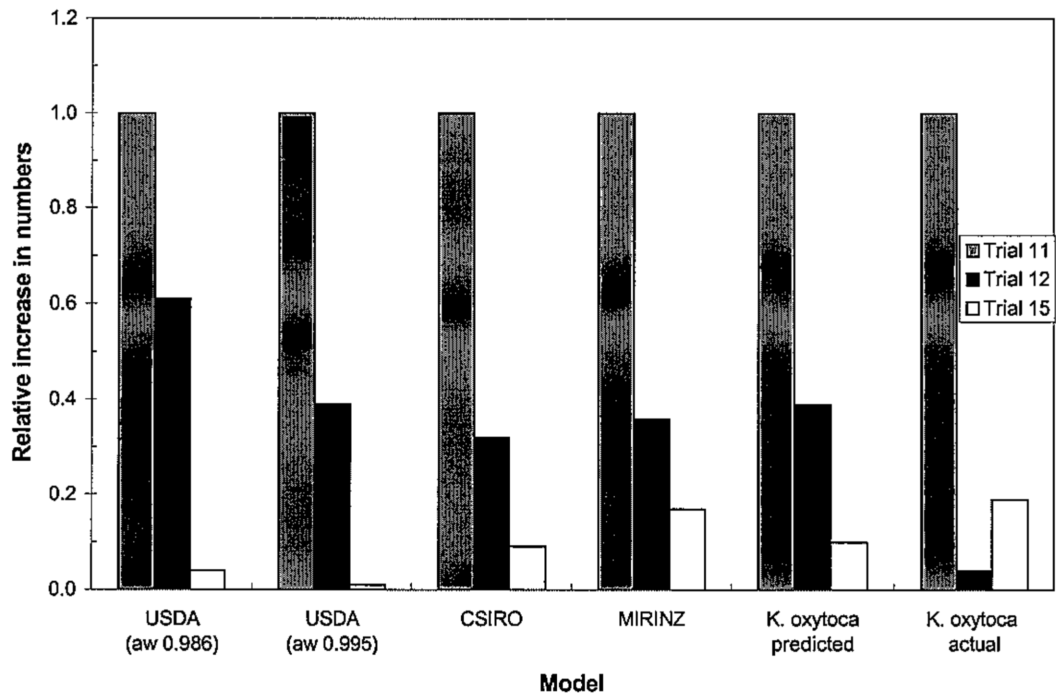


Figure 5: Relative increases during weekend chills - Cube roll

Effect of re-warming (hot gassing)

During several trials, including Trials 11, 12 and 15, for the final three hours of their time in the test chiller the sides were subjected to a rewarming phase.

Based on estimates from predictive models of potential increases in numbers which are summarised in Figure 7, a decision was made not to allow the surface temperature to rise higher than 20°C. In the event, on no occasion did the temperature rise to 18°C. For Trial 15 it did not reach 15°C. The gradual rises in surface temperature during the conditioning can be seen in Figures A11 to A15 in Appendix A. After weekend chilling under the current regime (Trials 1 to 4 - i.e. without a re-warming phase) plant management has often found it necessary to subject some carcases to a physical fat softening procedure. The sides subjected to the 'hot gas' conditioning during the trials did not require this treatment.

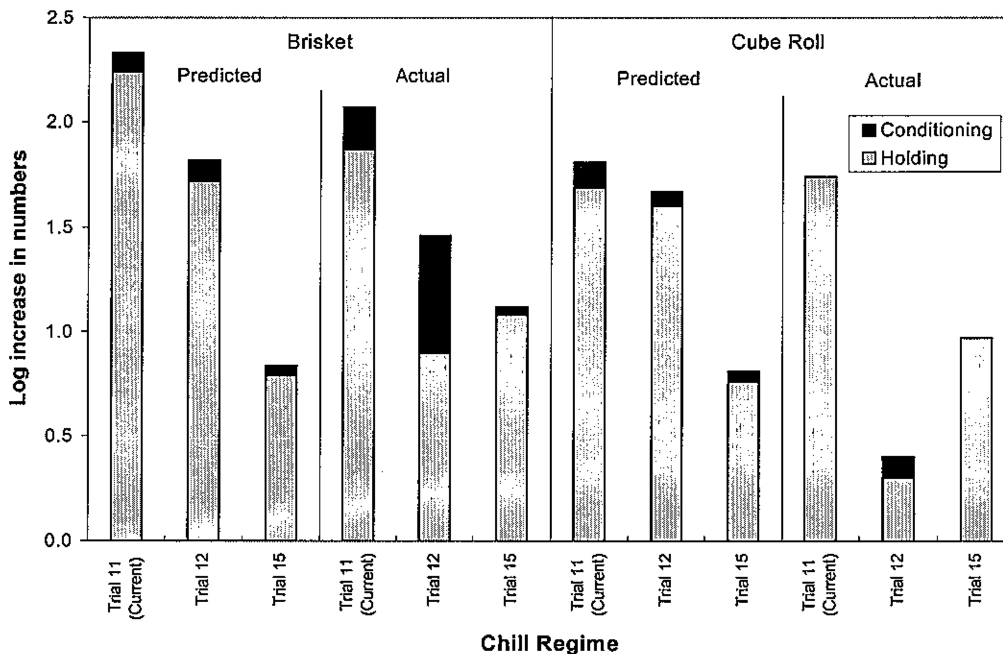


Figure 6: Effect of conditioning on growth of *E. coli* on the brisket and cube roll

The total increases in numbers of organisms and the proportions of those increases that are attributable to re-warming are presented in Figure 6 for each of Trials 11, 12, and 15. The figure also shows the increases predicted for each trial using the USDA model and the actual temperatures and water activities measured during the chilling. It can be seen that there is generally good agreement between the predicted values for *E. coli* and the actual increases in numbers of *K. oxytoca*.

It is apparent from Figure 7 that for the conditions to which the sides were subjected during the trials, the likely increase estimated using the prediction equations have a much smaller influence on any overall increase in numbers of *E. coli* than does the temperature of holding during the preceding period of 48 hours or so. Based on temperatures measured, during the period of rewarming the surface temperature increases to not more than 17.5°C near the end of the 3 hours. From the USDA model this would result in, at most, a 2-fold (0.3 log) increase.

From the prediction equations it has also been calculated that even if surface temperatures had reached 20°C and the period of conditioning had been as long as five hours, the expected increases would not have exceeded three-fold (0.5 log units).

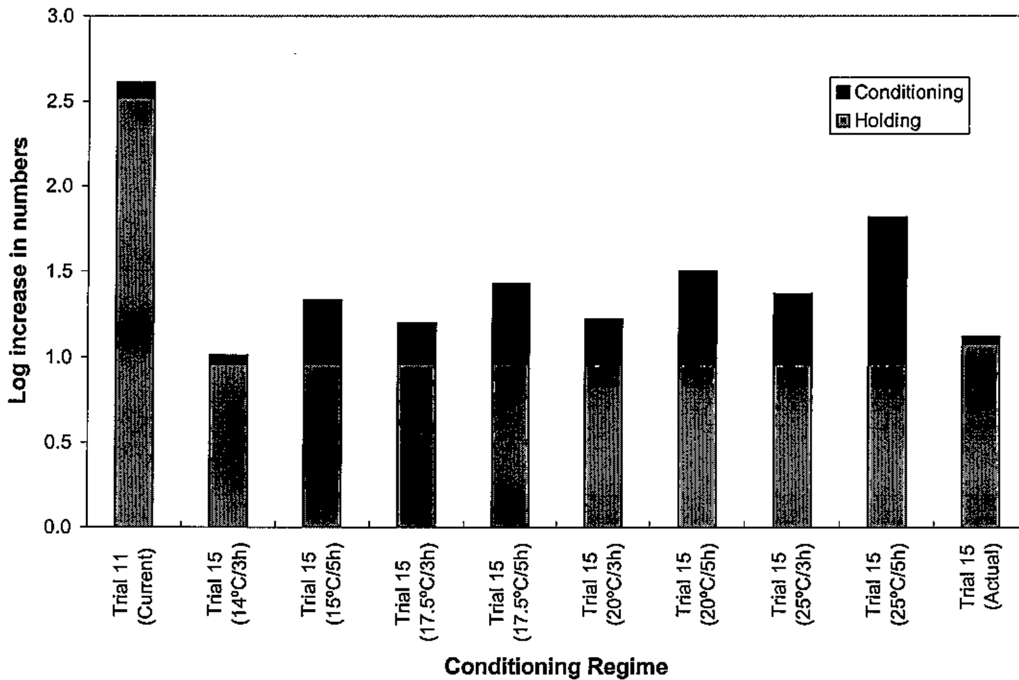


Figure 7: Predicted and actual increases in *E. coli* during various conditioning regimes

Conclusions

1. System control of the test chillers in the three participating plants was precise. With the exception of Trials 13 and 14, measured temperatures were very close to the set-point temperatures during modified chilling patterns which included a slight rise in temperature towards the end of the weekend to allow for some conditioning of the fat.
2. Holding the air temperature at 8 to 8.5°C then allowing it to rise to near 10°C during the final few hours of the weekend chill cycle reduced the problems of hard fat at boning.
3. Surface dryness, measured as water activity, followed a consistent pattern. There was a significant fall in water activity during the first hours of chilling followed by a gradual rise to a plateau after 20 to 30 hours. The plateau level for the brisket was consistently higher than that for the cube roll.
4. Computer models for growth of *E. coli* provide estimates of growth which agree well with the actual growth of a test organism, particularly the model which takes into account the surface water activity.
5. The use of the non-pathogenic organism *Klebsiella oxytoca* which has similar growth characteristics to *E. coli* provides a means of validating carcase chilling procedures.
6. The use of a heating cycle in the chiller for three to five hours prior to boning to soften the fat made only a small contribution to the total growth of the test organism over a weekend. Predictions from available models show that using a slightly lower holding temperature in combination with a conditioning and rewarming cycle results in less growth over the weekend than holding at near 10°C. The temperature rise which occurred during the rewarming was limited to around 8-9°C.
7. It is practicable to use a weekend chilling pattern which avoids holding beef sides for extended periods at a temperature at which significant growth of *E. coli* or other pathogens can occur. A pattern which uses a set-point temperature for the holding phase of 8°C followed by a period of 10 to 15 hours at 9.5°C will reduce the potential growth of *E. coli* to 10% or less of the growth possible at a holding temperature of 10°C.

Recommendations

The air temperature during the holding phase of weekend chilling should set at 8°C or below. If problems with fat hardness at boning are encountered, the air temperature should be allowed to rise 10°C for the final few hours of the holding phase. If this conditioning phase is not sufficient, it can be augmented by a cycle where the air is warmed by reverse cycle or electrical heating to 15°C to 20°C. This makes only a small contribution to the total weekend growth provided that the rewarming does not exceed five hours.

A recommended weekend chilling cycle is as follows:

Phase	Fan Speed	Temperature (°C)	Time (h)
Load	50% - 60%	10°C - 12°C	~2
Chill	100% (1.0 - 1.2m/s)	0°C - 5°C	8 - 12
Hold	40% - 50% (0.3 - 0.5 m/s)	7°C - 8°C	45 - 50
Equilibration	40% - 50% (0.3 - 0.5 m/s)	10°C	10 - 15
Conditioning (optional)	100%	15°C - 20°C	3

Recommended further work

1. The findings of this and other modules of Project MSQS.006 should be discussed with managements of export abattoirs where hard fat issues continue to cause difficulties and with AQIS.
2. Once there is sufficient feedback from these discussions, information packages should be prepared by MLA which describe the work carried out and which clearly indicate for export abattoirs the recommended procedures for safe beef side chilling.
3. The beneficial effect on inhibiting bacterial growth of closely controlling surface dryness should be further investigated. The relative humidity of chiller air is deliberately kept high in order to minimise carcase weight loss during chilling. However slight reductions in surface water activity may have a significant benefit on minimising bacterial growth without having a significant effect on weight loss.
4. During Project MSQS.006 the minimum holding temperature that management would permit was 8°C and the minimum duration of the conditioning period was 15 hours. Although the information presented in this report indicates that these conditions are very satisfactory, adoption of a holding temperature of 7°C would mean compliance with the ARMCANZ standard and possible further improvement in microbiological quality and it may be possible to achieve sufficient conditioning of hard fat by using a shorter period of conditioning than 15 hours. These investigations should be undertaken in the test chillers used for Project MSQS.006 because their characteristics are now well understood.

Appendix A - Temperature profiles for all runs

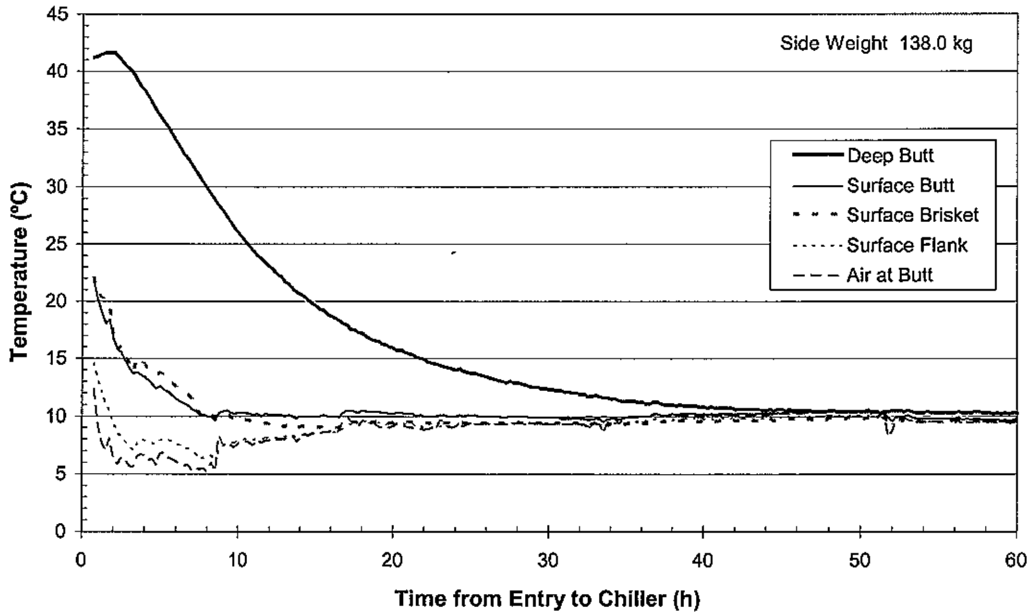


Figure A1: Trial 1 - Current pattern

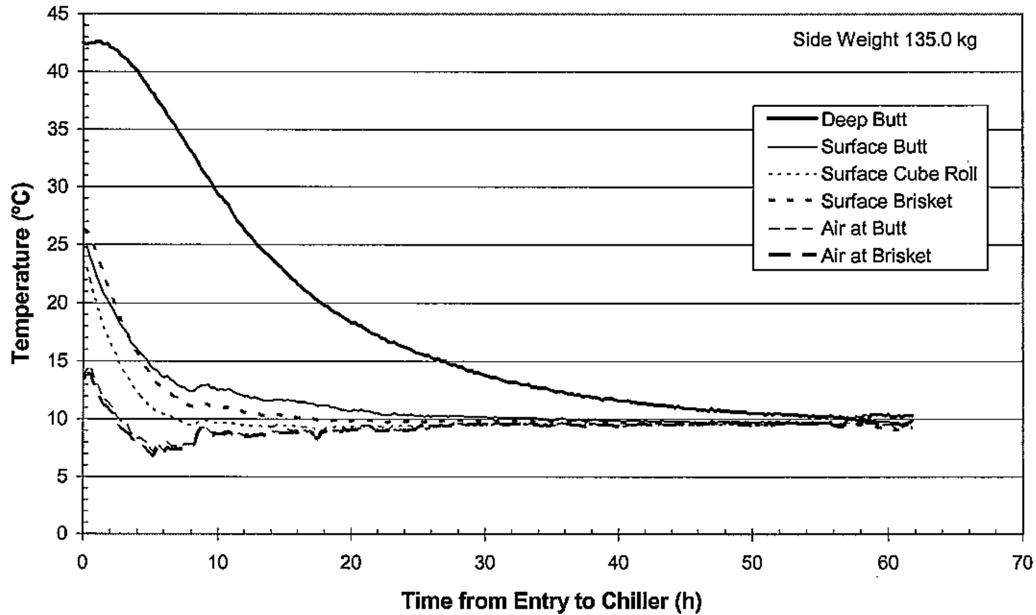


Figure A2: Trial 2 - Current pattern

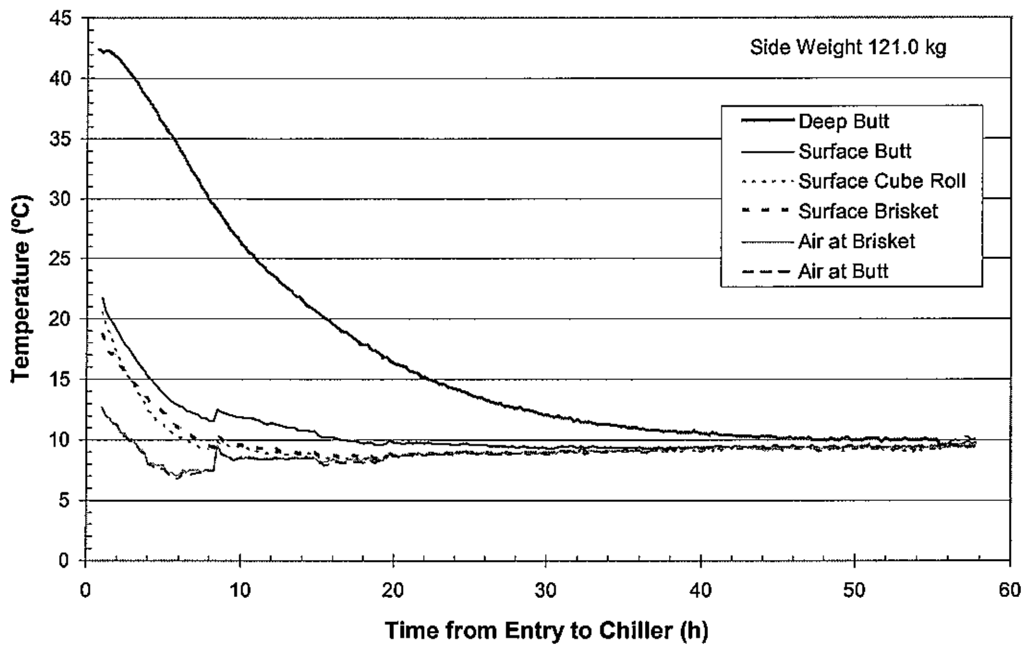


Figure A3: Trial 3 - Current pattern

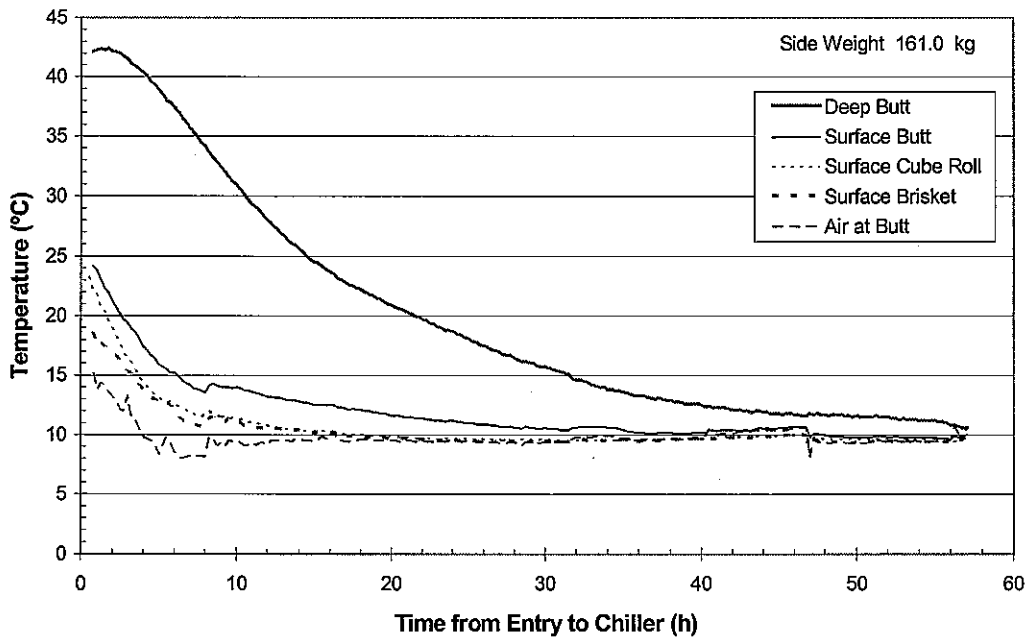


Figure A4: Trial 4 - Current pattern

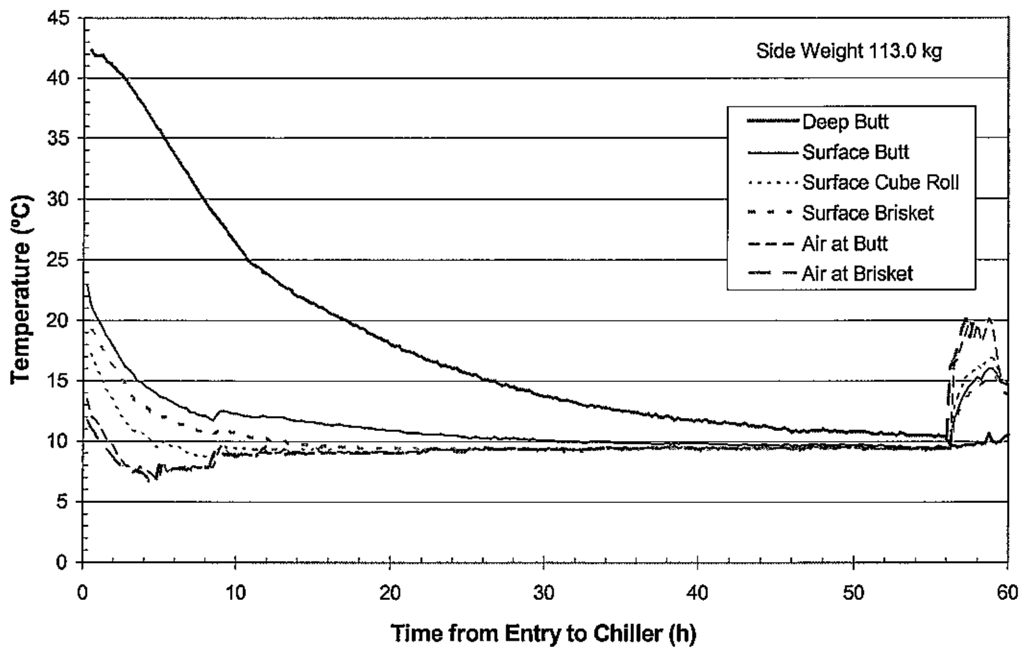


Figure A5: Trial 5 - Current pattern with hot gassing

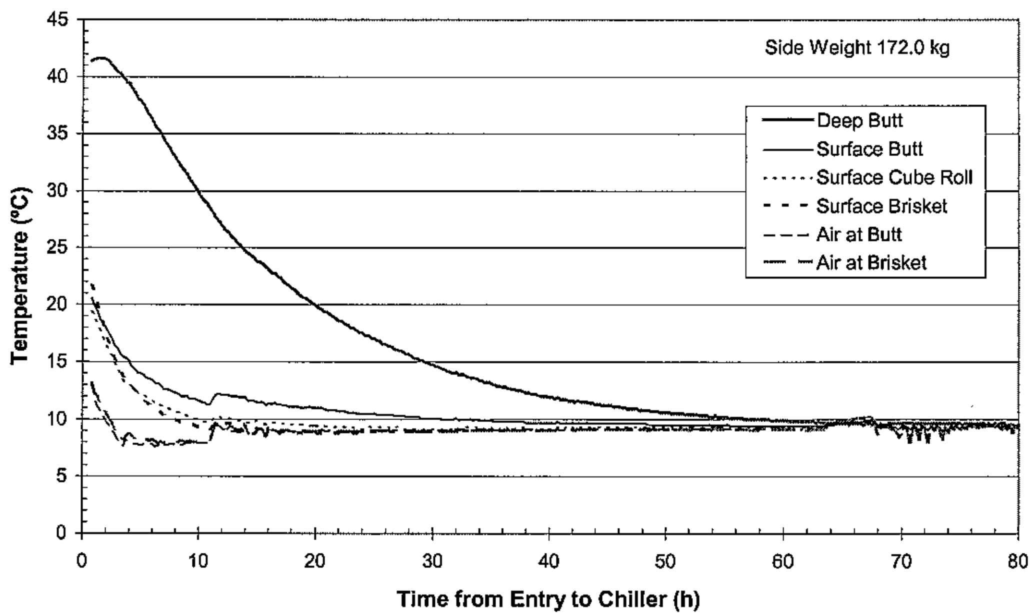


Figure A6: Trial 6 - Current pattern (long weekend)

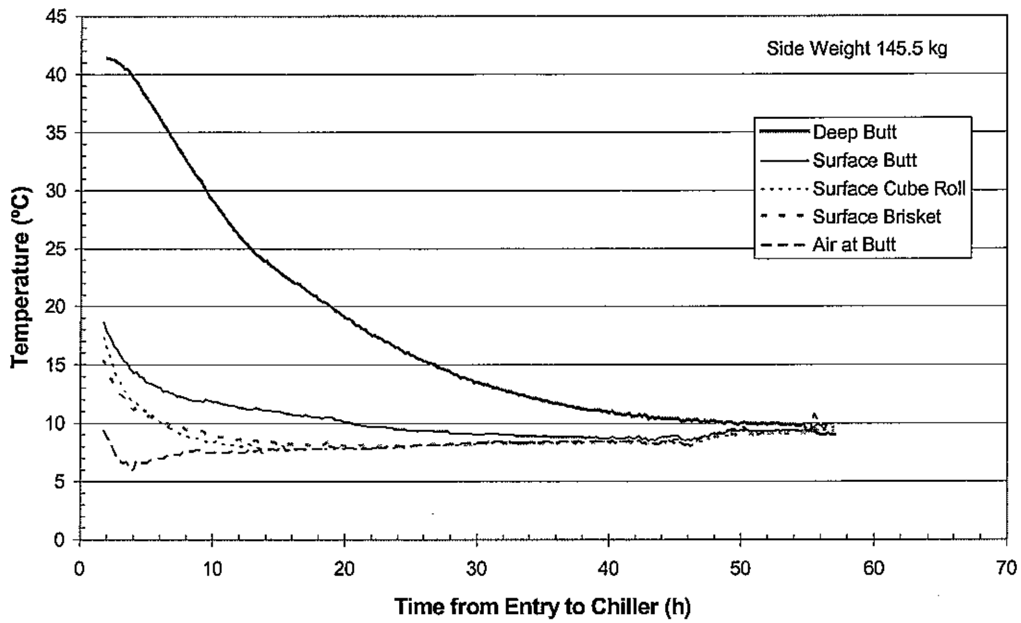


Figure A7: Trial 7 - Modified pattern

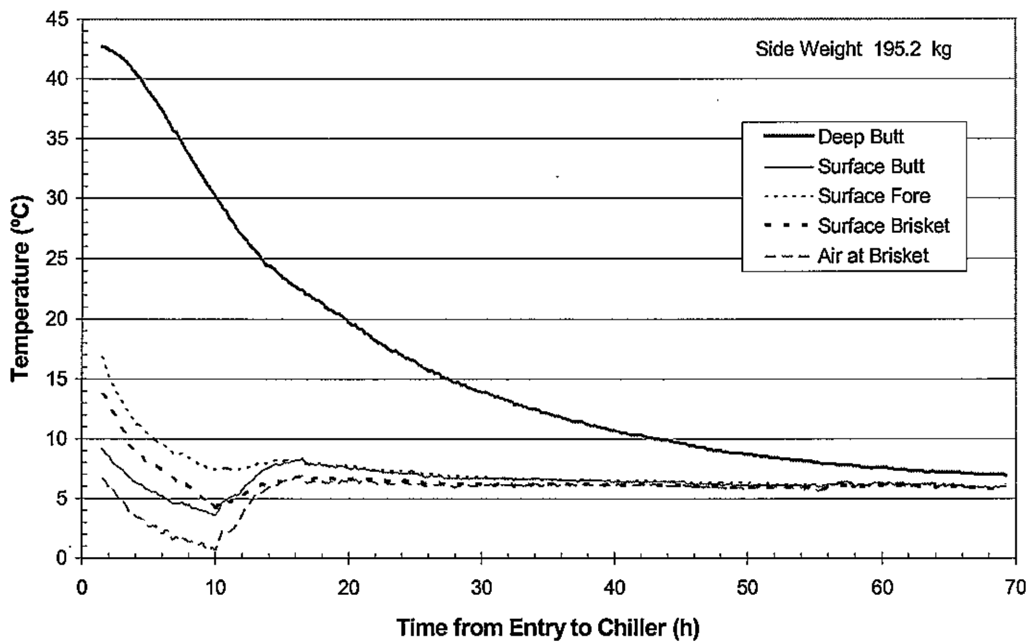


Figure A8: Trial 8 - Weekend pattern (Plant B)

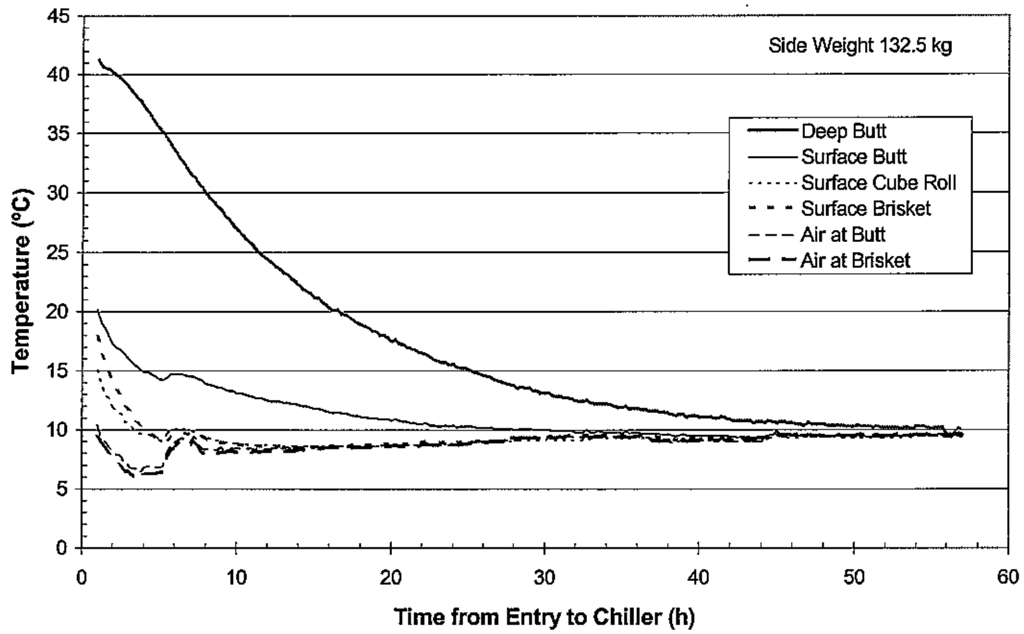


Figure A9: Trial 9 - Modified pattern

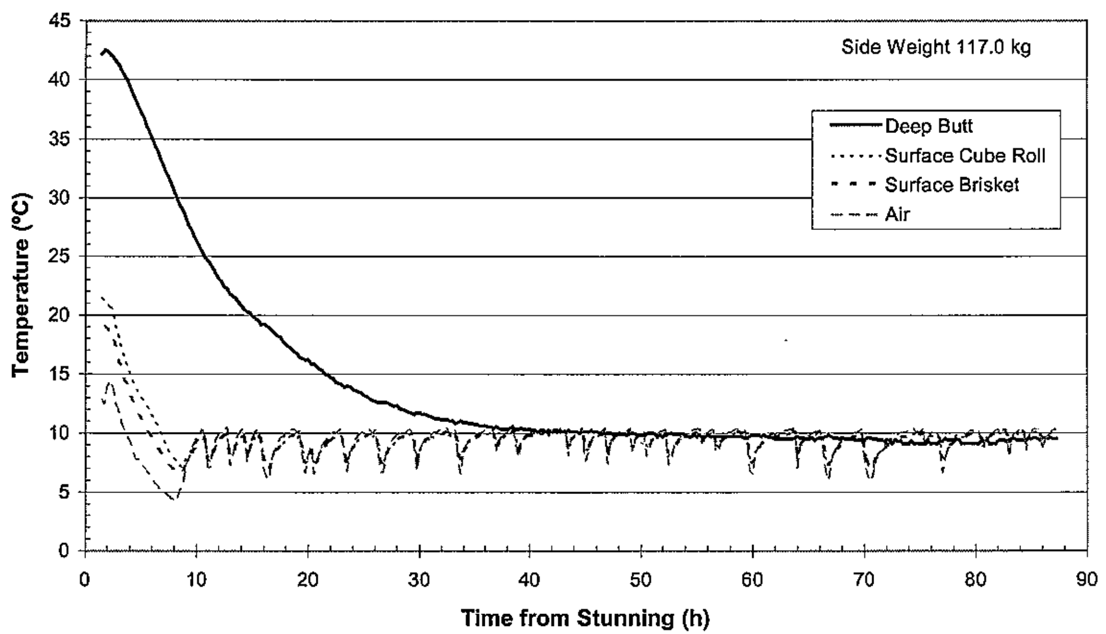


Figure A10: Trial 10 - Weekend pattern - Plant C (long weekend)

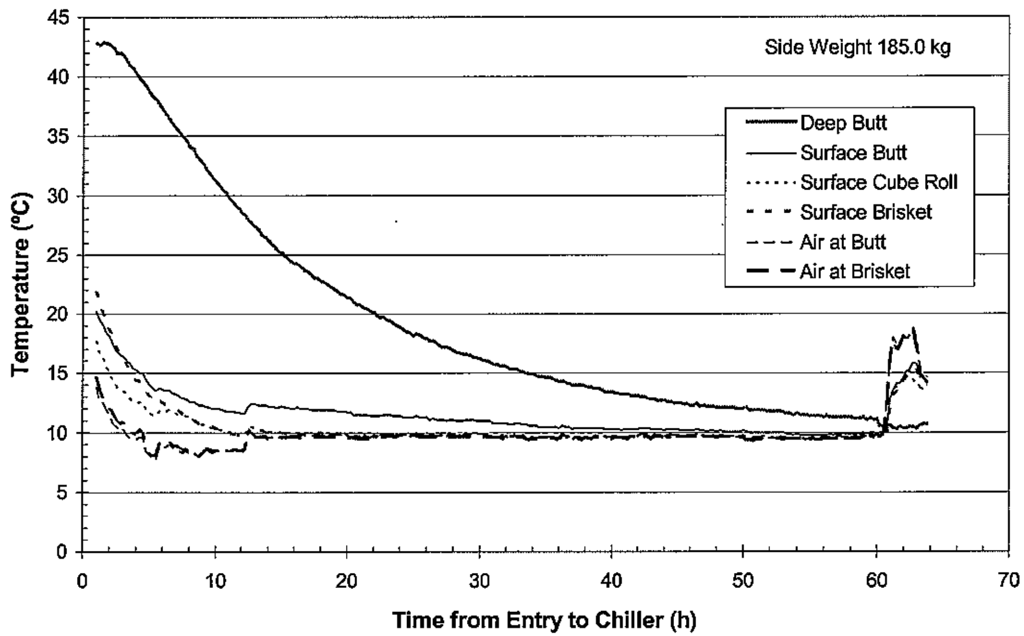


Figure A11: Trial 11 - Current weekend pattern with hot gas

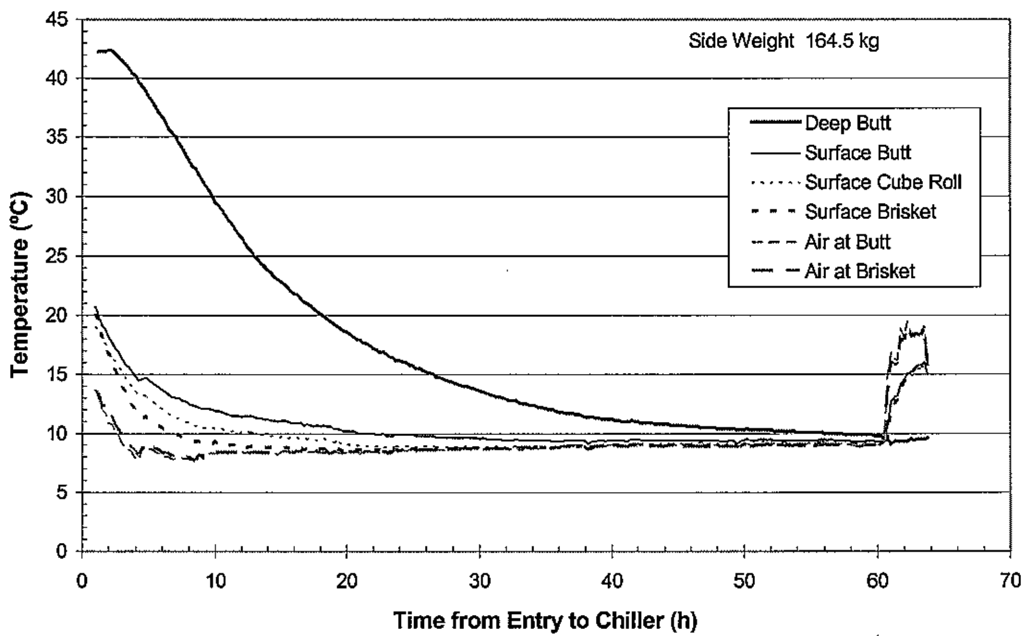


Figure A12: Trial 12 - Modified pattern with hot gas

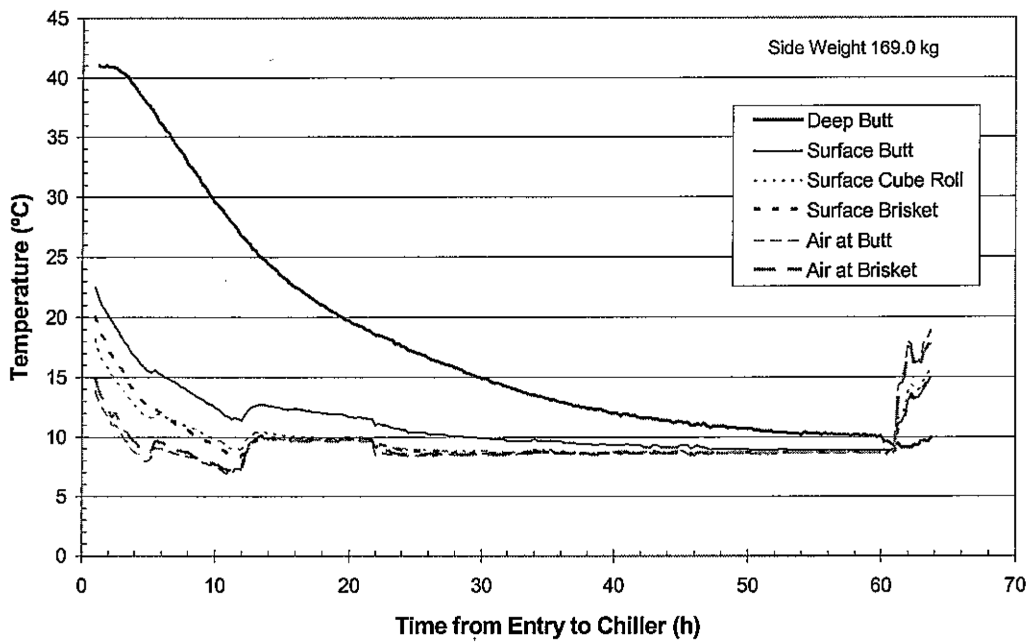


Figure A13: Trial 13 - Modified pattern

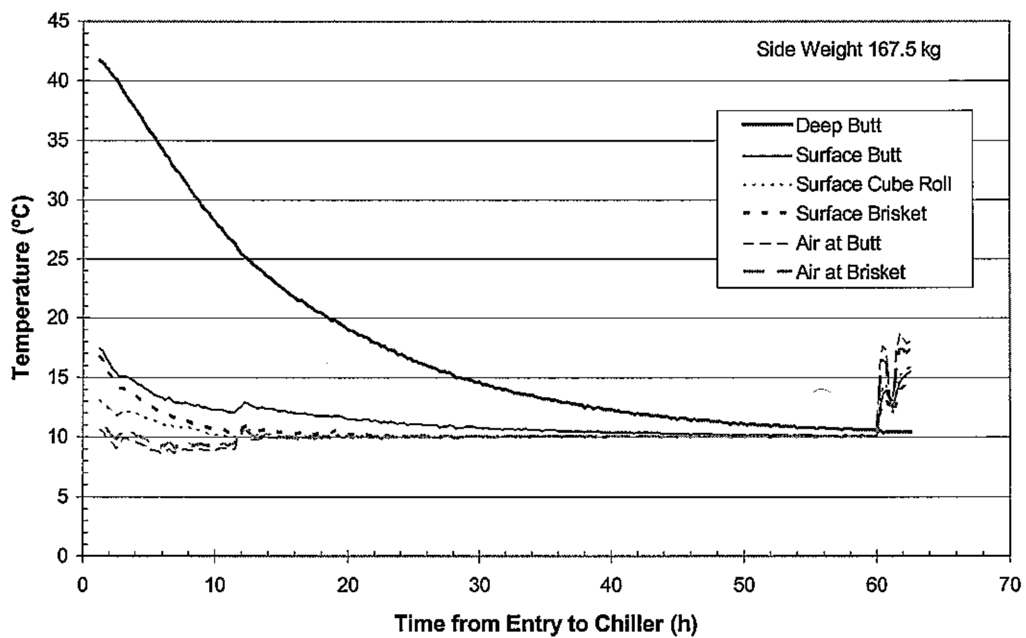


Figure A14: Trial 14 - Modified pattern

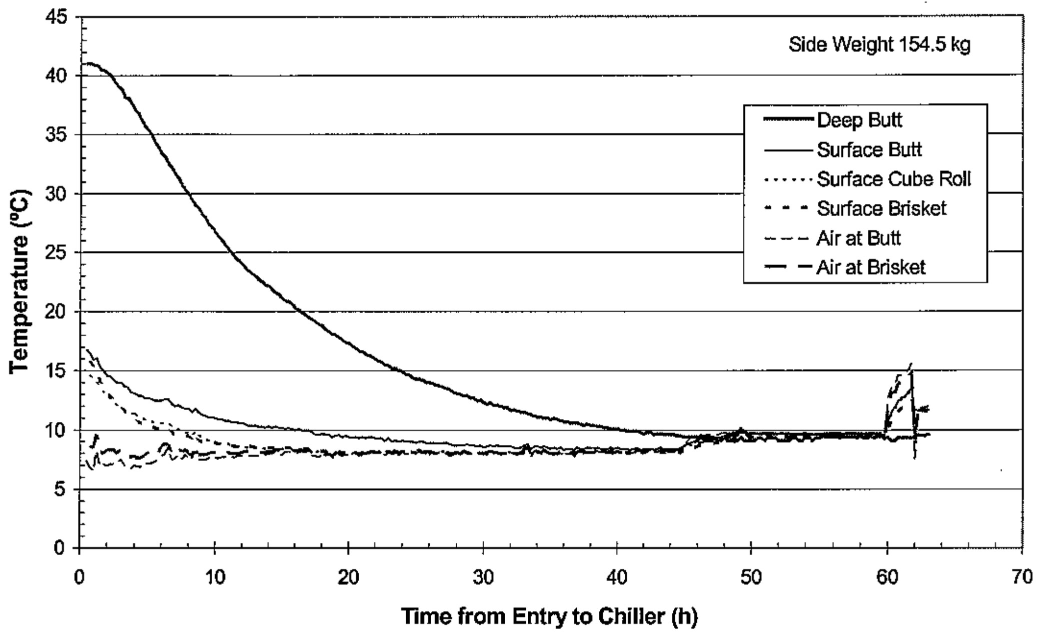


Figure A15: Trial 15 - Modified pattern

Appendix B - Comparison of the growth rates of *Klebsiella oxytoca* to that of *E.coli*

COMPARISON OF THE GROWTH RATES OF
KLEBSIELLA OXYTOCA* TO THAT OF *E.COLI

Division of Food Science and Technology
Brisbane Laboratory
Postal Address: P.O. Box 3312 Tingalpa DC, QLD, 4173.
Telephone :(07) 3214 2000 Fax: (07) 3214 2062

SUMMARY

Generation times for *E. coli* NCTC 9001 and *Klebsiella oxytoca* NRRL B-199 were determined at a range of temperatures (8 to 37^{0}C) normally encountered during carcass chilling. Similar growth rates for the two organisms were obtained at temperatures between 10 and 37^{0}C. At 8^{0}C growth was only observed for *K. oxytoca*. There was close agreement between this study and the work of Smith (1985), suggesting that the growth of *K. oxytoca* on meat would be similar to that obtained for *E. coli*. *K. oxytoca* may be suitable for use as a non-pathogenic marker organism in chiller assessments as required under Paragraph 5 (point 5.6) of Order 10 of the Export Meat Manual.

BACKGROUND

For an abattoir to adopt spray chilling for commercial use, the system must be assessed and approved by AQIS under Paragraph 5 (point 5.6) of Order 10 of the Export Meat Manual. This order states that a program must be undertaken to assess the effectiveness of spray chilling in controlling microbial growth. The most accurate form of assessment is through challenge tests using non-pathogenic test organisms with similar growth characteristics to common pathogens i e. *E. coli* and *Salmonella*.. Currently there are no organisms approved in Australia for use in challenge tests to assess chilling programs.

Klebsiella. oxytoca is non-pathogenic and has similar responses to heat, organic acids and antimicrobial agents as *E. coli* and *Salmonella*. It has been used to assess antimicrobial treatments in the United States by the FSIS, and was recently approved by AQIS for the evaluation of a commercial hot water decontamination system installed at Oakey Abattoir.

CSIRO was contracted by AMT to evaluate and compare the growth rates of *K. oxytoca* and *E. coli* at various temperatures, to determine if *K. oxytoca* may be suitable for use as an indicator for *E. coli* growth on carcasses during chilling.

METHODOLOGY

Growth rates of *K. oxytoca* and *E. coli* were determined at 5^0, 8^0, 10^0, 15^0, 25^0, 32^0 and 37^0C. Cultures of both *K. oxytoca* NRRL B-199 and *E. coli* NCTC 9001 were prepared using the same procedure. Cultures were grown in 125 ml side-arm flasks containing 20 ml of tryptone soya broth (TSB, Oxoid) for approximately 20 h at 37^0C. Flasks were not shaken. Approximately 2 ml of the overnight culture was inoculated into 20 ml of TSB to a cell density of 10 Klett units (Klett-Summerson photoelectric colourimeter). The culture was re-incubated for approximately 2 h at 37^0C until a cell density of 50 Klett units was reached.

Each culture was diluted to approximately 2 x 10^3 cfu/ml in two 20 ml amounts of TSB. The cultures were immersed in water baths set at 5^0, 8^0, 10^0, 15^0, 25^0, 32^0 and 37^0C. At each temperature approximately 10 samples were withdrawn at regular intervals over the incubation period. Aliquots of 0.1 ml of appropriate dilutions were spread-plated onto Tryptone Soy Agar (Oxoid) supplemented with 0.2% glucose and 0.2% yeast extract (TYSG). Numbers of bacteria were enumerated after incubation at 37^0C for 24 hours.

Growth rates were calculated using the model of Baranyi (1993) and the square root of the generations/hr plotted against temperature according to the method of Ratkowsky *et al* (1982).

RESULTS

The growth curves for *K. oxytoca* and *E. coli* cultures incubated at 37^0, 32^0, 25^0, 15^0, 10^0, 8^0 and 5^0C are shown in Appendix 1, while the counts are given in Appendix 2. Each point in the figures represents the arithmetic mean of the $\log_{10}$ of the number of cells per ml enumerated in each of the duplicate cultures tested at each time point.

The growth curves for *E. coli* and *K. oxytoca* grown at 37^0, 32^0, 25^0 and 15^0C (Figures 1 to 4) were similar. The growth rates at 10^0C (Fig 5) were also similar although *E. coli* appeared to have a longer lag at this temperature. *E. coli* did not begin growing at 10^0C until 23 and 30 hours after inoculation, compared to only 9 hours for *K. oxytoca*. No increase in numbers was recorded for *E. coli* after 121 hours incubation at 8^0C (Figure 6), with numbers reaching 4 logs only after 220 hours. At the same temperature numbers of *K. oxytoca* increased between 46 and 121 hours, reaching 5.2 $\log_{10}$ cfu/ml and further increased to 7.6 $\log_{10}$ cfu/ml after 220 hours.

A second experiment at 8^0C (8a in Table 1) was conducted in an endeavour to shorten the lag times observed in the first experiment. The inoculums for this experiment were grown at 10^0C to try and adapt the organisms to the low incubation temperature. The inoculums were diluted into TSB and incubated at 8^0C, counts were obtained as for the first experiment. In this trial numbers of *E. coli* decreased after 69 hours incubation while *K. oxytoca* grew at a rate of one generation every 12.05 hours with numbers reaching 8.08 $\log_{10}$ cfu/ml after 189 hours.

Numbers of *E. coli* incubated at 5^0C (Figure 7) gradually decreased by 1 log over the incubation period (269 hours). There was no change in the numbers of *K. oxytoca* recorded over the same period.

The comparison of growth rates of *E. coli* and *K. oxytoca* is shown in Table 1. The generation times were calculated using models developed by Baranyi et al (1993).

Table 1: Growth rates of *E. coli* and *K. oxytoca*

Incubation Temperature (^{0}C)	Generation Times (hr)		Generations per hour	
	E. coli	*K. oxytoca*	*E. coli*	*K. oxytoca*
37	0.31	0.37	3.19	2.70
32	0.40	0.44	2.50	2.27
25	0.65	0.64	1.54	1.56
15	2.36	2.40	0.42	0.42
10	8.43	7.04	0.12	0.14
8	35.10	12.53	0.03	0.08
8a	NG	12.05	NG	0.08
5	NG	NG	NG	NG

NG = no growth

The square root of the generations per hour was plotted against temperature (Figure 8) using the method of Ratkowsky et al (1982). There was little difference between the growth rates of the two bacteria at the temperatures used in this trial.

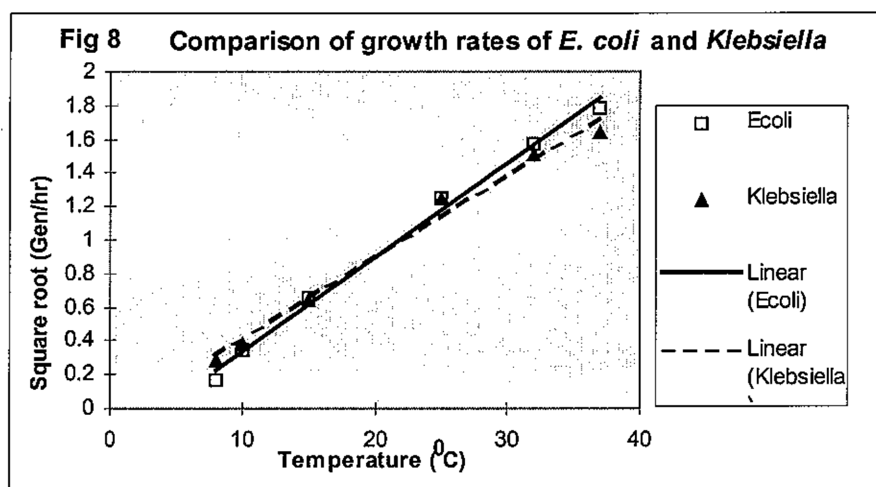


Fig 8 Comparison of growth rates of *E. coli* and *Klebsiella*

DISCUSSION

Growth rates for *K. oxytoca* NRRL B-199 and *E. coli* NCTC 9001 were similar between 10^0C and 37^0C. The results are similar to *E. coli* growth rates on meat reported by Smith (1985). This indicates that *K. oxytoca* would behave similarly to *E. coli* on meat and carcase surfaces, at least over the range of temperatures usually encountered during the first 24 h of chilling (37^0C to 10^0).

There was a significant difference in growth rates between *E. coli* and *K. oxytoca* grown at 8^0C (35.1 h and 12.5 h respectively). Though not investigated in this present study it is possible that *E. coli* NTCT 9001 may be extremely sensitive to temperatures below 10^0C. Smith (1985) calculated the generation time of *E. coli* SF (isolated from sheep faeces) to be 17.2 hours at 8.2^0C. This is closer to the generation time of *K. oxytoca* (12.5 hr) found in this present study at 8^0C.

The purpose of the present work was to investigate the suitability of *K. oxytoca* as a marker for *E. coli* on chilled carcases. Based on the findings presented here *K. oxytoca* can be used as a marker for *E. coli* growth at temperatures ranging between 10 and 37^0C. The results obtained at 8^0C indicate that it may not be appropriate to use *K. oxytoca* as an indication of *E. coli* at this temperature,. although comparisons with other studies would indicate that this is a possibility. Given that the growth of *K. oxytoca* at 8^0C was approximately 12 h it is unlikely that any significant growth would occur in the initial 24 h of chilling. However, over long chilling periods, such as weekends, significant growth of *K. oxytoca* may occur at temperatures that prevent the growth of *E. coli*. Since this errs on the side of safety it should not preclude the use of *K. oxytoca* as a non-pathogenic marker organism in chiller assessments as required under Paragraph 5 (point 5.6) of Order 10 of the Export Meat Manual.

CONCLUSIONS

1 Data obtained from this study indicate growth rates of *E.coli* and *Klebsiella* are similar at temperatures recorded during chilling on beef carcases

2 The close agreement of data from this study and that of Smith suggest that *Klebsiella oxytoca* NRRL B-199 may be a suitable organism for use as an indicator of possible *E.coli* growth on beef carcases.

3 *Klebsiella oxytoca* NRRL B-199 may be suitable for use as a non-pathogenic marker organism in chiller assessments as required under Paragraph 5 (point 5.6) of Order 10 of the Export Meat Manual.

RECOMMENDATIONS

* That AMT apply to AQIS to use *Klebsiella oxytoca* NRRL B-199 as a non - pathogenic marker organism in chiller assessments as required under Paragraph 5 (point 5.6) of Order 10 of the Export Meat Manual.

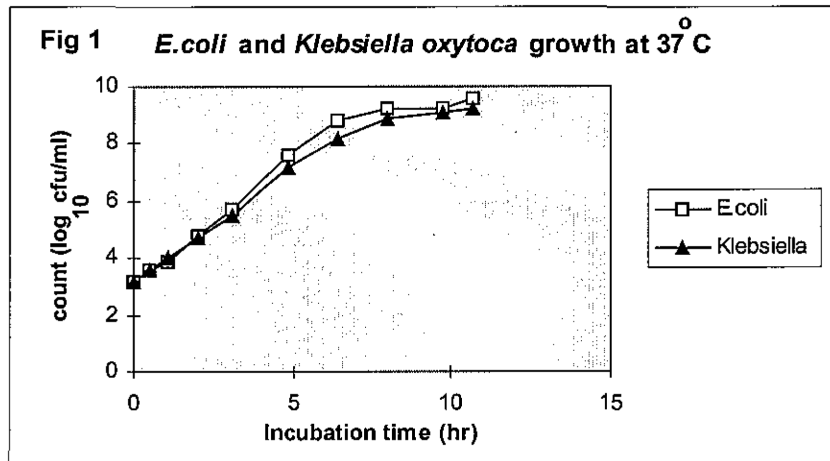
REFERENCES

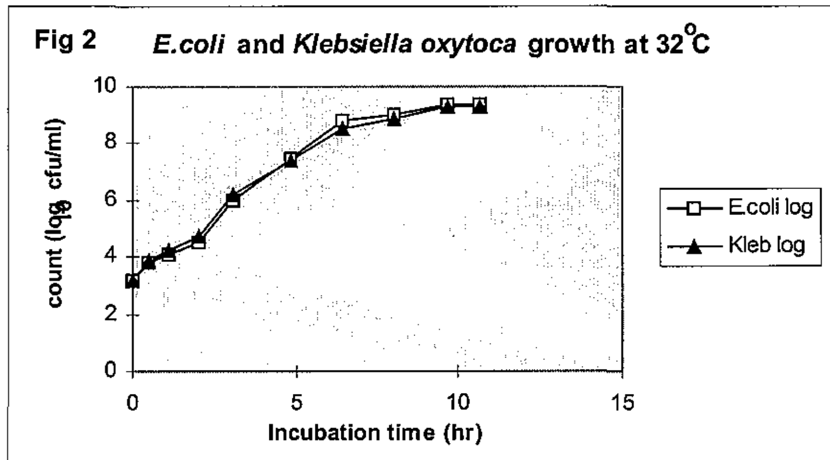
Baranyi, J., T. A. Roberts, and P. McClure (1993). A non-autonomous differential equation to model bacterial growth. Food Microbiol., 10 (1), 43-59.

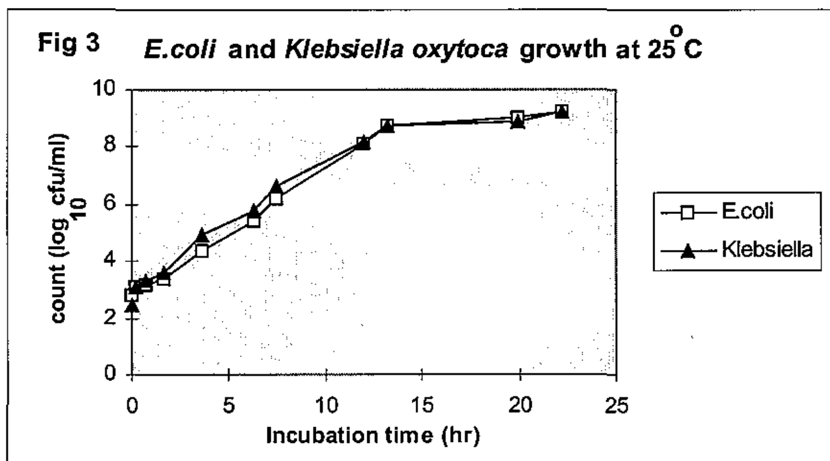
Ratkowsky, D.A., Olley, June, McMeekin, T, A and Ball, A. (1982). Relationship between temperature and growth rate of bacterial cultures. J. Bacteriol. 149, 1-5.

Smith, M. G.(1985). The generation time, lag time, and minimum temperature of growth of coliform organisms on meat, and the implications for codes of practice in abattoirs. J. Hyg. 94, 289-300.

APPENDIX 1







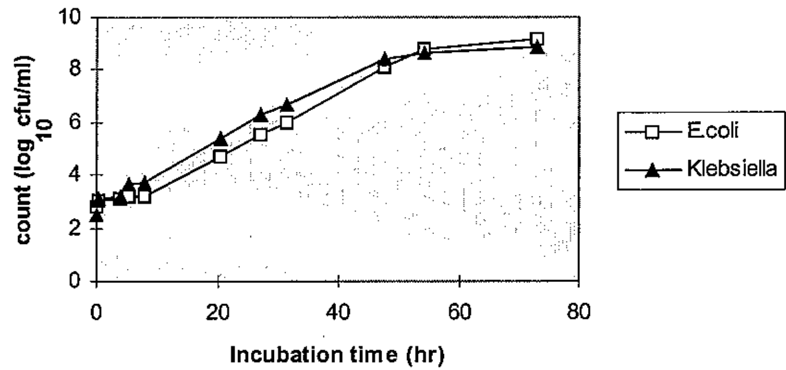


Fig 4 E.coli and Klebsiella oxytoca growth at 15 °C
count (log10 cfu/ml)
10
8
6
4
2
0
0
20
40
60
80
Incubation time (hr)
E.coli
Klebsiella

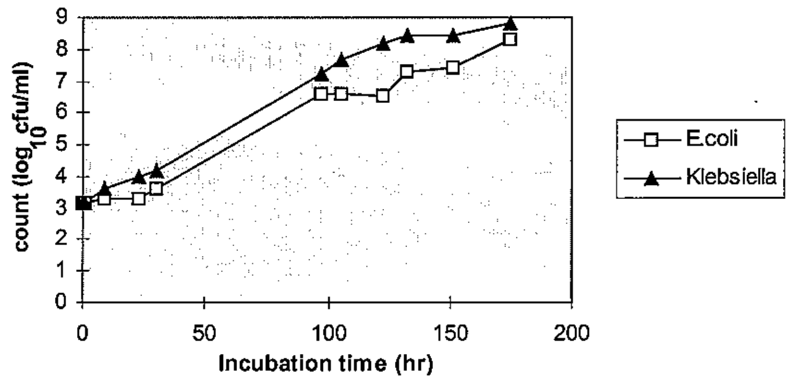


Fig 5 E.coli and Klebsiella oxytoca growth at 10 °C
count (log10 cfu/ml)
9
8
7
6
5
4
3
2
1
0
0
50
100
150
200
Incubation time (hr)
E.coli
Klebsiella

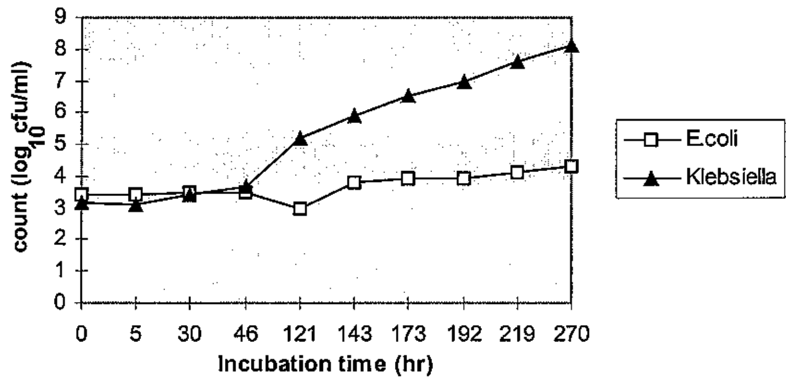


Fig 6 E.coli and Klebsiella oxytoca growth at 8 °C
count (log10 cfu/ml)
9
8
7
6
5
4
3
2
1
0
0 5 30 46 121 143 173 192 219 270
Incubation time (hr)
E.coli
Klebsiella

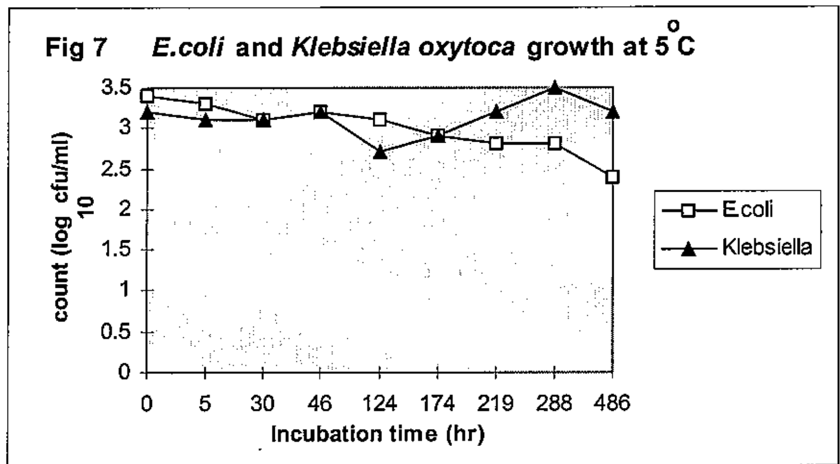


Fig 7 ***E.coli*** **and** ***Klebsiella oxytoca*** **growth at 5°C**

APPENDIX 2

Table 1: Growth of E. coli and Klebsiella oxytoca at 37^{0}C

37^{0}C			
E. coli		*K. oxytoca*	
incubation time (hr)	**Count average log/ml**	**incubation time (hr)**	**Count average log/ml**
0	3.2	0	3.2
0.50	3.6	0.50	3.6
1.08	3.9	1.08	4.0
2.00	4.8	2.00	4.7
3.08	5.7	3.08	5.5
4.83	7.6	4.83	7.2
6.42	8.8	6.42	8.2
8.00	9.2	8.00	8.9
9.70	9.2	9.70	9.1
10.66	9.6	10.66	9.2

Table 2: Growth of *E. coli* and *K. oxytoca* at 32^{0}C

32^{0}C			
E. coli		*K. oxytoca*	
incubation time (hr)	**Count average log/ml**	**incubation time (hr)**	**Count average log/ml**
0	3.22	0	3.31
1.00	3.78	1.00	3.87
1.50	4.11	1.50	4.18
2.15	4.54	2.15	4.70
4.00	6.00	4.00	6.20
6.00	7.54	6.00	7.39
8.00	8.77	8.00	8.47
10.00	9.04	10.00	8.92
12.00	9.38	12.00	9.30
13.58	9.42	13.58	9.31

Table 3: Growth of *E. coli* and *K. oxytoca* at 25⁰C

25⁰C			
E. coli		*K. oxytoca*	
incubation time (hr)	Count average log/ml	incubation time (hr)	Count average log/ml
0	2.8	0	2.5
0.17	3.1	0.17	3.1
0.75	3.2	0.75	3.3
1.65	3.4	1.65	3.6
3.63	4.4	3.63	4.9
6.3	5.4	6.3	5.8
7.47	6.2	7.47	6.6
12.00	8.1	12.00	8.2
13.22	8.7	13.22	8.7
19.97	9.0	19.97	8.9
22.18	9.2	22.18	9.2

Table 4: Growth of *E. coli* and *K. oxytoca* at 15⁰C

15⁰C			
E. coli		*K. oxytoca*	
incubation time (hr)	Count average log/ml	incubation time (hr)	Count average log/ml
0	2.8	0	2,5
0.42	3.0	0.42	3.1
4.0	3.1	4.0	3.2
5.33	3.2	5.33	3.6
7.83	3.2	7.83	3.7
20.42	4.7	20.42	5.4
27.25	5.5	27.25	6.3
31.50	6.0	31.50	6.7
47.57	8.1	47.57	8.4
54.33	8.8	54.33	8.6
73.17	9.2	73.17	8.9

Table 5: Growth of *E. coli* and *K. oxytoca* at 10^0C

10^{0}C			
E. coli		*K. oxytoca*	
incubation time (hr)	**Count average log/ml**	**incubation time (hr)**	**Count average log/ml**
0	3.2	0	3.2
1.08	3.2	1.08	3.2
9.17	3.3	9.17	3.6
23.00	3.3	23.00	4.0
29.83	3.6	29.83	4.2
97.33	6.6	97.33	7.2
105.25	6.6	105.25	7.7
122.25	6.5	122.25	8.2
132.50	7.3	132.50	8.4
151.42	7.4	151.42	8.4
174.42	8.3	174.42	8.8

Table 5: Growth of *E. coli* and *K. oxytoca* at 8^0C

8^{0}C			
E. coli		*K. oxytoca*	
incubation time (hr)	**Count average log/ml**	**incubation time (hr)**	**Count average log/ml**
0	3.4	0	3.2
5.08	3.4	5.08	3.1
29.5	3.5	29.5	3.4
46.08	3.5	46.08	3.7
121.0	3.0	121.0	5.2
142.83	3.8	142.83	5.9
173.42	3.9	173.42	6.5
192.30	3.9	192.30	7.0
219.83	4.1	219.83	7.6
269.25	4.3	269.25	8.1

Table 7: **Growth of *E. coli* and *K. oxytoca* at 5^0C**

5^0C			
E. coli		*K. oxytoca*	
incubation time (hr)	**Count average log/ml**	**incubation time (hr)**	**Count average log/ml**
0	3.4	0	3.2
5.33	3.3	5.33	3.1
29.75	3.1	29.75	3.1
46.33	3.2	46.33	3.2
123.66	3.1	123.66	2.7
174	2.9	174	2.9
219.17	2.8	219.17	3.2
288.33	2.8	288.33	3.5
486.08	2.4	486.08	3.2