



final report

Project code:	A.MFS.0117
Prepared by:	Dr Heather Haines / Ms Joanne Bobbitt Department of Primary Industries Victoria
Date submitted:	May 2008

PUBLISHED BY Meat & Livestock Australia Limited Locked Bag 991 NORTH SYDNEY NSW 2059

Validation of the "Food Spoilage Predictor" model in red meat

Meat & Livestock Australia acknowledges the matching funds provided by the Australian Government and contributions from the Australian Meat Processor Corporation to support the research and development detailed in this publication.

This publication is published by Meat & Livestock Australia Limited ABN 39 081 678 364 (MLA). Care is taken to ensure the accuracy of the information contained in this publication. However MLA cannot accept responsibility for the accuracy or completeness of the information or opinions contained in the publication. You should make your own enquiries before making decisions concerning your interests. Reproduction in whole or in part of this publication is prohibited without prior written consent of MLA.

Abstract

The Middle East markets are key markets for Australian chilled lamb, airfreighted as chilled carcases or sea freighted as vacuum-packaged product. Ensuring the performance of the products in these markets is essential.

This project aimed to define the shelf life limiting organisms under the current packaging system, using information from broth and shelf life simulations to adapt models for the prediction of shelf life in this vacuum packaged product. Effective predictive models for spoilage will help industry provide realistic shelf life for their product, and reduce wastage.

Pseudomonas spp. are the predominant spoilage species of aerobically stored meat. The "Food Spoilage Predictor" model for spoilage developed at the University of Tasmania has been validated in minced beef and chilled pork. It has not, however, been previously validated for use with carcase or whole muscle red meats.

The validated model will provide a powerful tool for the Australian red meat industry to predict product performance in domestic and export markets.

Executive summary

The Middle East markets are key markets for Australian chilled lamb, airfreighted as chilled carcases or sea freighted as vacuum-packaged product. Ensuring the performance of the products in these markets is essential.

This project aimed to define the shelf life limiting organisms under the current packaging system, using information from broth and shelf life simulations to adapt models for the prediction of shelf life in this vacuum packaged product. Effective predictive models for spoilage will help industry provide realistic shelf life for their product, and reduce wastage.

Pseudomonas spp. are the predominant spoilage species of aerobically stored meat. The "Food Spoilage Predictor" model for spoilage developed at the University of Tasmania has been validated in minced beef and chilled pork. It has not, however, been previously validated for use with carcase or whole muscle red meats.

The validated model will provide a powerful tool for the Australian red meat industry to predict product performance in domestic and export markets.

The objective of the project was to validate the use of the 'Food Spoilage Predictor' model for the prediction of aerobic (*Pseudomonas* spp) spoilage of red meats.

The predicted generation times for *Pseudomonas* are consistent with observations on meat. The results suggest that a model for *Pseudomonas* growth on carcase meat should include terms to account for pH and lactic acid conditions that are sub-optimal for *Pseudomonas* growth.

Acknowledgements

The authors gratefully acknowledge the contribution of Associate Professor Tom Ross, University of Tasmania, the collaborating Victorian sheep meat exporter, and Mr Stuart Brough, Maersk Line for their contribution to the project.

Contents

		Page
1	Background5	
2	Project Objectives5	
2.1 2.2	Project Objective	
3	Methodology6	
3.1 3.2 3.3 3.4	Cold chain monitoring6Microbiological assessment of product through simulated shipment and storage in the market7Gas flush options to extend shelf life7Predictive Microbiology7	
4	Results and Discussion8	
4.1	Results	
4.1.1	Cold chain monitoring8	
4.1.2	Microbiological assessment of product through simulated shipment and storage in the market	
4.1.3	Gas flush options to extend shelf life 19	
4.1.4	Predictive microbiology	
5	Success in Achieving Objectives	
6	Conclusions20	
7	Bibliography21	
8	Appendices23	
8.1 8.2	Appendix 1: Predictive microbiology	

1 Background

The Victorian government funded "Naturally Victorian Initiative" (NVI) has worked to address market access issues for fresh food products from Victoria into the Singapore market, and the project has been extended to address potential shelf life issues identified in the Middle East market for chilled lamb.

The Middle East markets are key markets for Australian chilled lamb, airfreighted as chilled carcases or sea freighted as vacuum-packaged product. Ensuring the performance of the products in these markets is essential. As part of the NVI project temperature data on the seafreight cool chain for vacuum packaged product was collected, to identify any problem areas in the cool chain. The project aimed to define the shelf-life limiting organisms under the current or any new packaging systems, and use information from broth and shelf life simulations to adapt or develop models for the prediction of shelf life in this vacuum packaged product.

The project was extended, with MLA support, to validate predictive models more appropriate for use with airfreighted product. *Pseudomonas* spp. are the predominant spoilage species of aerobically stored meat, and the "Food Spoilage Predictor" model for spoilage was developed at the University of Tasmania in 1991 (Ross and McMeekin 1991; Neumeyer, Ross et al. 1997). The model has been validated in chilled pork (Widders 1993; Widders, Coates et al. 1994) and used extensively to establish market access for Australian pork in Singapore (Coates 2000). The model has been validated in trials in milk and minced beef (Neumeyer, Ross et al. 1997), but has not been validated for use with carcases or whole muscle meats. Validation of the model would provide a powerful tool for the Australian red meat industry to predict product performance in domestic and export markets. Effective predictive models for spoilage will help the industry to provide realistic shelf life for their product, and reduce wastage.

2 **Project Objectives**

2.1 Project Objective

The objective of this project was to validate the use of the "Food Spoilage Predictor" model for aerobic (*Pseudomonas* spp.) spoilage of red meats.

2.2 Additional details

The project used laboratory based studies with known inocula of spoilage *Pseudomonas* spp. to test the model.

Temperature logs collected from airfreight of carcase sides were used to simulate larger laboratory based studies, to both validate the model and predict the shelf life of product in the market under current cool chain conditions.

3 Methodology

3.1 Cold chain monitoring

In conjunction with a Victorian sheep meat exporter, the supply chain into the Middle East was mapped. Data logs from shipments were collected and assessed to identify any issues with the cool chain that may be common to all Australian lamb supply chains to the Middle East. To assist with this the following 'logger form' was developed for the processor to use

	Time	Date	Logger ID	Logger ID	Logger ID	Door			
Time logger								 	
placed into							/	 	
boxes	<u> </u>					- /			
Loggers position	on in conta	iner							
	te the appro	oximate pos	silion of the log	igers in the box	marking the				
			-		\rightarrow				
Time on									
truck									
			-						
I ime									
received at									
wiidii									
Time loaded			-						
to ship									
Time arrived									
in export									
market									
Time loaded									
onto truck									
Time loaded									
to chiller									

3.2 Microbiological assessment of product through simulated shipment and storage in the market.

The ideal simulation would be conducted on a full seafreight container, but this is expensive and results in considerable waste disposal issues. Advice received from the National Reefer Coordinator for Maersk Line was that the temperature and air flow would more accurately represent a full container when cardboard is placed along the floor of the container, leaving the end of the T-floor (just inside the doors) clear. Maersk International provided a 20 foot reefer which was loaded as shown below and held at -1.5°C, to simulate a typical shipment of 24 days, after which product was transferred to 4° C.



Product was assessed microbiologically on day 0 (loading the container) and subsequently at weekly intervals over a total of 77 days of storage post slaughter.

Microbiological assessment was in accordance with Australian Standard and AOAC methods, and conducted in a NATA accredited laboratory (Accreditation number 14349). Tests conducted were:

- Total Viable Count (TVC) (variation of AS5013.1-2004 incubating at 25°C),
- Pseudomonasspp. (AS5013.21-2004),
- Lactic acid bacteria (LAB) (De Man et al(1960))
- Brochothrix spp. (AS5013.22-2004), and
- *E. coli* and coliform (AOAC 991.14) in the latter stages of the trial only.

Replicate 10 gram samples (3) of each of the two products were tested throughout the shelf life trial; these were vacuum packaged bone-in and boneless legs (AS1766.3.1-1991). An empirical assessment of the organoleptic quality of the product (appearance, odour and drip) was made at each testing interval.

3.3 Gas flush options to extend shelf life.

The Victorian processor expressed an interest in using carbon monoxide (CO) as part of the gas flushing. The use of this gas was investigated, in terms of regulatory approval in the region and efficacy.

3.4 Predictive Microbiology

The project aimed to validate the aerobic spoilage model "Food Safety Predictor" (developed by the University of Tasmania) for use with lamb. We also sought to develop spoilage models for vacuum packaged product.

4 Results and Discussion

4.1 Results

4.1.1 Cold chain monitoring

The cool chain for the Victorian processor was similar to the generic red meat value chain shown in Figure 1. However, for this chain the product underwent transhipment in Dubai, where it was maintained on power until shipped to Qatar.

Disposable loggers were provided by TN'T¹ for these trials, though fewer logs of shipments from Victoria to the Middle East were obtained than was anticipated (Figures 2-5). Inadequate information was retrieved regarding the key events during shipment and therefore it was not possible to interpolate these on the time-temperature data logs.

Additional logs were provided from another lamb processor. The data logs obtained are provided in Figures 6 and 7, followed by an explanation of the events during shipment.

¹ <u>http://www.profox-intl.com/logistics_management.php</u>

Figure 1: Lamb value chain²



² Adapted from "The impact of credence attributes on value chain operations", from the **Our Rural Landscape** Initiative project *Profitable and Sustainable Paths to Market*. (State of Victoria, 2004)



Figure 2: Temperature log of Shipment of chilled vacuum packaged lamb from Victoria to the Middle East



Figure 3: Temperature log of Shipment of chilled vacuum packaged lamb from Victoria to the Middle East (Dec 2006).



Date/Time

Figure 4: Temperature log of Shipment of chilled vacuum packaged lamb from Victoria to the Middle East (Jan 2007).



Figure 5: Temperature log of Shipment of chilled vacuum packaged lamb from Victoria to the Middle East (Aug 2007).



Figure 6: Temperature log of Shipment of chilled vacuum packaged lamb Australia to the Middle East (June 2007).

Data	-	•••	A
08/06/07	15.24	24.51	Unit activated
08/06/07	21.24	03.68	Container entered Terminal
09/06/07	15.24	01.70	Container was 'Load Full'.
10/06/07	02.54	00.97	33.50 hours have elapsed since unit was activated
12/06/07	04.54	00.00	First time records 00.00 C
12/06/07	08.39	00.03	Temperature is <00.00 C for the first time
30/06/07 During this tim 8 days, was lo	02.54 e the co aded or	-00.03 ontainer was dia nto second carr	Temperature has remained below 00.00 C for ~18 days. scharged at Malaysia. The container sat on the terminal for ier and discharged at Jebel Ali Dubai, UAE.
30/06/07 hours earlier.	03.09	00.00	Container was discharged at Jebel Ali Dubai, UAE 1.5
02/07/07	13.54	-00.03 C	Temperature constant for next 3 days.
05/07/07 period the con	14.54 tainer w	-00.07 vas loaded on b	Temperature fluctuates for the next 4.5 days. During this board the third carrier
10/07/07	07.24	00.00	Container was discharged full at Damman, Saudi Arabia
11/07/07 Damman, Sau	01.24 Idi Arab	00.63 ia, the tempera	From the time the container was discharged at ture rose to 00.63 C.
11/07/07 00.28 C and th	01.54 nen fluc	00.59 tuates between	For the next 24 hours the temperature falls to 00.24 C and 00.42 C for the next 30 hours.
13/07/07 reaching a low	06.39 v of 00.0	00.21 03 C.	For the next 42 hours the temperature decreases,
14/07/07 between 00.07	21.24 7 C and	00.07 00.10 C.	For the next 8 hours the temperature fluctuates
15/07/07 17/07/07. Duri 16.26 hours, 1	06.09 ng this 5/07/07	00.14 period the conta '.	Temperature increases reaching 04.94 C at 01.54 hours, ainer was delivered from Damman Terminal, Saudi Arabia,
17/07/07 From this time	02.09 the ten	03.54 nperature fluctu	Temperature decreases reaching 01.39 C ~5 hours later. lates from 00.80 C to 04.44 C
17/07/07	17.24	36.84	Digital temperature recorder was retrieved



Date/ti

ime



Date	Time	C	Assessment
04/06/07	10.56	03.16	Unit is activated.
05/06/07			Container received at Australian terminal
06/06/07	04.26	00.97	Temperature records <01.00 C for first time.
09/06/07			Container loaded onto vessel
12/06/07	08.11	00.21	~8 days after the container is loaded the container reaches
its lowest reco	rded tei	mperature of 00	0.21 C
18/06/07	19.11	00.52	From this point in time the temperature does not fall rs the temperature sequences slowly rise to reach 00.90 C
below 00.52 C	5. For th	e next 105 hou	
at 04.41 hours	5. 23/06/	⁄07.	
23/06/07 reach this poir 23/06/07.	04.56 nt. The t	00.97 emperature the	The temperature rises 00.07 of a degree Celsius to en reaches a high of 04.93 C, 16 hours later at 20.56 hours,
23/06/07	20.56	04.93	The temperature starts to decrease. Almost 7 days 52 C, at 16.41 hours, 30/06/07.
later the tempe	erature	recorded is 00.	
04/07/07	08.41	00.63	The temperature increases reaching 02.00 C, 8 hours later point in time it takes 48 hours for the temperature to once
at 16.56 hours	, 04/07/	/07. From this p	
again register	00.63 C	2.	
08/07/07			Container in Jeddah, Saudi Arabia
09/07/07	06.26	00.66	Temperature increases to 02.29 C (13.11 hours, 09/07/07), g a low of 00.94 C at 04.26 hours, 11/07/07.
before it starts	to drop	again, reachin	
11/07/07	06.41	00.97	From this point in time the temperature inside the
container rises	s rapidly		
12/07/07	01.26	32.57	Recorder removed from container

4.1.2 Microbiological assessment of product through simulated shipment and storage in the market

The results of microbiological assessment of bone-in and boneless vacuum packaged lamb legs are presented in Figures 8 and 9 respectively.

Figure 8: Microbiological shelf life trial over 77 days, vacuum packaged bone-in lamb legs.



Figure 9: Microbiological shelf life trial over 77 days, vacuum packaged boneless lamb legs.



Note the limit of detection of the pour plate methods (TVC and LAB) was 5cfu/g, and for the spread plate methods (*Pseudomonas* and *Brochothrix*) was 50cfu/g.

As can be seen in Figure 8 the total viable count (TVC) of the bone-in product gradually increased throughout the trial, as expected, regardless of the temperature. The total count was at 'marginal' levels (according the the Meat Standards Committee guidelines) by day 42. Although the spoilage bacteria did not reach spoilage levels, the total count remains above 'marginal' levels by day 56.

Pseudomonas spp. remained below the limit of detection of the test from the bone-in product for the majority of the trial. This organism was detected on days 21 and 24, during simulated shipment and on days 42 and 56, during refrigerated storage (Figure 8). However, the counts were below spoilage levels.

Brochothrix spp. remained at low levels for most of the simulated shipment, and was detected at levels below spoilage day 24 (Figure 8).

Lactic acid bacteria (LAB) numbers remained essentially unchanged for the duration of the trial, at undetectable levels(Figure 8).

Figure 9 shows the TVC of the boneless product increased throughout the trial, as expected, regardless of the temperature (-2.0°C then 4.0°C). The total count was approaching 'marginal' levels (according the theMeat Standards Committee guidelines) by day 24. Although the spoilage bacteria did not reach spoilage levels, the total count remains above the 'marginal' level by day 35.

Pseudomonas spp. remained below the limit of detection of the test for the majority of the trial (Figure 9). This organism was detected on days 7 and 24, during simulated shipment and on days 42 and 49, during refrigerated storage. However, the counts were below spoilage levels.

Brochothrix spp. remained at low levels for most of the simulated shipment, and was detected at levels below spoilage day 24 (Figure 9).

Lactic acid bacteria numbers of these organisms remained essentially unchanged for the during the simulated shipment phase of the trial, at undetectable levels (Figure 9). These levels were maitained until day 42 (approximately 2.5 weeks refrigerated storage). Lactic acid bacteria increased in number to day 63 (approximately 5.5 weeks refrigerated storage), though remaining well below spoilage levels. The numbers of this organism returned to undetectable levels on days 70 and 77. This is unusual as the numbers would be expected to remain at levels observed on day 63, if not increase in number.

Lactic acid bacerial Isolates were recovered from both the bone-in (day 7, simulated seafreight) and boneless (days 42, 49 and 56, simulated refrigerated storage) product. The isolates, 8 in total, were 16S sequence analysed at the University of Tasmania. Initial results from the 'Ribosomal Database Project"³ revealed that 5 of the 8 isolates were recognised as LAB; *Lactobacillus* spp., *Leuconostoc* spp., *Carnobacterium* spp.

In general, the two products performed with respect to organoleptic quality. The odour, colour and texture of both products did not alter significantly throughout the shelf life trial period. However, it was noted that the vacuum packaging did stick to the meat surface on days 0-28 for bone-in products and on days 0-35 for boneless product. Boneless product sampled on day 35 was reported to have 'drip' evident in the packs.

4.1.3 Gas flush options to extend shelf life

Preliminary discussions with the Victorian value chain indicated that they were seeking to use carbon monoxide (CO) as part of modified atmosphere packaging (MAP) to improve the performance of the sheep meat in the market.

The use of CO in MAP storage of pork has long been accepted in Norway (Sørheim, Aune et al. 1997; Sørheim, Nissen et al. 1999), wherein the gas (usually 0.4%) is incorporated into a MAP mix with carbon dioxide (CO₂) and nitrogen (N₂). The CO₂ levels range from 30 - 60% and have antimicrobial activity, while the N₂ is inert and included to prevent pack collapse.

The USDA FSIS³ Directive 7120.1 accepts CO as a "Safe and suitable ingredient[s] used in production of meat and poultry products" at concentrations less than 0.3 - 0.4% in specifically defined packaging systems for case ready muscle and ground meat. While the use of CO in MAP in Australia is not widespread, it is listed in as a permitted processing agent in FSANZ Standard 1.3.3, "at a level necessary to achieve a function in the processing of the food –".

The use of CO in MAP has been used for the packaging of retail packs. The focus for this project was on primal meat cuts under vacuum packaging. Trials incorporating carbon monoxide as part of the modified atmosphere packaging gas mix for primal cuts, though discussed with Food Science Australia (FSA), were not conducted.

4.1.4 Predictive microbiology

Refer to Appendix 1 for evaluation of the 'Food Spoilage Predictor' (*Pseudomonas* spp.) model in aerobically stored lamb.

As a result of the low numbers of Lactic acid bacteria recovered from either the bone-in or boneless product during this trial, it was not possible to develop a spoilage model for red meat under vacuum packaging.

5 Success in Achieving Objectives

The objective of the project was to validate the use of the 'Food Spoilage Predictor' model for the prediction of aerobic (*Pseudomonas* spp) spoilage of red meats.

The predicted generation times for *Pseudomonas* are consistent with observations on meat. The results suggest that a model for *Pseudomonas* growth on carcase meat should include terms to account for pH and lactic acid conditions that are sub-optimal for *Pseudomonas* growth.

6 Conclusions

The predicted generation times for *Pseudomonas* are consistent with observations on meat. The results suggest that a model for *Pseudomonas* growth on carcase meat should include terms to account for pH and lactic acid conditions that are sub-optimal for *Pseudomonas* growth.

³ United States Department of Agriculture Food Safety Inspection Service

7 Bibliography

Anon, AOAC Official Method 991.14 Coliform and Escherichia coli Counts in Foods

Anon. AS 1766.3.1-1991 Examination of specific products-Meat and meat products other than poultry.

Anon, AS5013.1-2004 Food Microbiology - Examination for specific organisms-Standard plate count

Anon, AS5013.21-2004 Food microbiology - Meat and meat products - Enumeration of Pseudomonas spp

Anon, AS5013.22-2004 Food microbiology - Meat and meat products - Enumeration of Brochothrix thermosphacta - Colony-count technique

Bobbitt, J. L., S. Barlow, et al. (1997). <u>Extension of shelf-life of fresh pork for the export market</u>. 43rd International Congress of Meat Science and Technology, Auckland New Zealand, ICOMST.

Coates, K. (2000). Trial shipment of chilled pork to the Singapore market. Melbourne, Victorian Institute of Animal Science: 58.

Coates, K. J. (2002). Validation of the cold chain for pork into Singapore. Melbourne, Australia, Department of Natural Resources and Environment.

Coates, K. J., J. C. Beattie, et al. (1995). "The contribution of carcase contamination and the boning process to microbial spoilage of aerobically stored pork." <u>Food Microbiology</u> **12**: 49-54.

Coates, K. J., L. Kamperman, et al. (1997). <u>The use of predictive microbiology to improve the shelf-life of fresh pork.</u> Manipulating Pig Production VI, Canberra, ACT, Australasian Pig Science Association.

J. C. De Man, M. Rogosa, M.E Sharpe (1960) A medium for the cultivation of lactobacilli Journal of Applied Microbiology **23**(1): 130–135.

Neumeyer, K., T. Ross, et al. (1997). "Development of a predictive model to describe the effects of temperature and water activity on the growth of spoilage pseudomonads." <u>International Journal of Food Microbiology</u> **38**: 45-54.

Neumeyer, K., T. Ross, et al. (1997). "Validation of a model describing the effects of temperature and water activity on the growth of psychrotrophic pseudomonads." <u>International Journal of Food</u> <u>Microbiology</u> **38**: 55-63.

Ross, T. and T. A. McMeekin (1991). "Predictive Microbiology. Applications of a square root model." <u>Food Australia</u> **43**: 202 - 207.

Widders, P. R. (1993). Applying predictive microbiology for improved marketing of fresh pork. Melbourne, Victorian Institute of Animal Science, Pork Research and Development Corporation.

Widders, P. R., K. J. Coates, et al. (1994). <u>Use of predictive microbiology to extend the shelf-life</u> <u>of fresh pork at retail outlets.</u> Australian Society for Microbiology Annual Scientific Meeting, Melbourne, Australia, Australian Society for Microbiology.

8 Appendices

8.1 Appendix 1: Predictive microbiology



School of Agricultural Science

Food Microbiology Group[†]

Evaluation of Food Spoilage Predictor (Pseudomonas spp.) Model in Aerobically Stored Lamb. (MLA Project A. MFS.0117)

Interim Final Report (November 2007)

> prepared by Tom Ross for

Primary Industries Research Victoria

[†] In August 2007, the partners of the Australian Food Safety Centre of Excellence agreed that, unless projects were jointly initiated and undertaken, the individual organizations would be responsible for all aspects of those projects.

Project A. MFS.0117

Section A. Evaluation of Pseudomonas spp. model.

Introduction

As part of project A. MFS.0117, psychrotrophic pseudomonads were enumerated on lamb carcasees stored aerobically at 4°C or 8°C. The growth kinetics were modelled and compared to that predicted by "Pseudomonas Predictor" a mathematical model developed at University of Tasmania with joint funding from the former Meat Research Corporation, and Pork and Dairy Research Corporations. The model and its development are described in Neumeyer *et al.* (1997a, b).

The Pseudomonas model includes the effect of temperature and water activity only, i.e. does not include terms for pH or lactic acid concentration, which may be relevant to growth potential of pseudmonads on aerobic stored lamb carcasees.

The model had previously been validated for a range of foods including dairy products and minced red meat. The purpose of this work was to evaluate the model's applicability to carcase meats.

Methods and Materials

Four data sets were available for fitting, each including triplicate measurements of Pseudomonads at up to 25 intervals. Two sets of data for storage at 4°C and at 8°C were assessed. One data set for each temperature had initial numbers of psychrotrophic pseudomonads in the range $3 - 4 \log CFU.g^{-1}$ while the other set had lower initial pseudomonad counts, in the range 1.5 to 2 $\log CFU.g^{-1}$.

The data were fitted to a modification of the Logistic (or 'autocatalytic') function, due to Dalgaard (1993), that enables the logistic function to include a delay before initiation of microbial growth. The model is of the form:

$$\operatorname{Log} N_{(t)} = \operatorname{Log} \left| \begin{array}{c} A + \frac{D}{1 + \exp\left\{\frac{0.693}{t_{G_{\min}}}(M - t)\right\}} \end{array} \right|$$
Eqn. A.1

where A is the initial number of psychrotrophic pseudomonad per gram on the product, D is the final number of psychrotrophic pseudomonas cells per gram that is achieved, $t_{G_{min}}$ is the minimum generation time of the psychrotrophic pseudomonads, and M is the time at which the number of psychrotrophic pseudomonads is half the maximum density that is finally achieved, (i.e. D/2).

Experimental data were fitted to Equation A.1 by minimisation of the root mean square of residuals (RMSE) using the Solver routine of Microsoft ® Excel. For the 8°C, low inoculum dataset, two anomalously low observation at the last sampling time were removed from the dataset prior to fitting of Eqna. A.1, but had little effect on the fitted parameters. The model allowed the lag time, generation time, and maximum population density to be estimated objectively. These estimates were compared to those from the Pseudomonas predictor model assuming that the water activity of the meat was 0.992.

Results

Comparison of the data and the fitted models are presented in Figures A.1 - 4. Summary data describing the fitted growth curves are presented in Table A.1



Figure A.1 Growth of Pseudomonads on aerobically stored lamb at 4°C. Squares are observations; the curve is the data fitted to Eqn. A.1.





Figure A.2 Growth of Pseudomonads on aerobically stored lamb at 4°C. Squares are observations; the curve is the data fitted to Eqn. A.1.

Figure A.3a Growth of Pseudomonads on aerobically stored lamb at 8°C. Squares are observations; the curve is the data fitted to Eqn. A.1.

In Figure A.2a, it is evident that there is much scatter in the data. Moreover, the predicted growth rate (steepest slope on the fitted curve) is dominated by only two sets of data and appears anomalously fast. Other, equally plausible, growth curves might be able to be fitted to the data. To explore this, the generation time in Eqn. A.1 was fixed to the value expected from the Pseudomonas Predictor model. Other parameters of Eqn. A.1 were then fitted by non-linear regression using Solver. Finally, Solver was used to then estimate the generation time once the 'best-estimates' of the other parameters of Eqn. A.1 were fitted. The resulting fitted growth curve is shown in Figure A3.b. The goodness of fit (RMSE value; see Table A.1) by this latter method is only marginally lower than the original fitting.



Figure A.3b Growth of Pseudomonads on aerobically stored lamb at 8°C and fitted to Eqn. A.1 using a different method involving initial constraints on the fitted values. Squares are observations; the curve is the data fitted to Eqn. A.1.



Figure A.4 Growth of Pseudomonads on aerobically stored lamb at 8°C. Squares are observations; the curve is the data fitted to Eqn. A.1.

Generation time and lag time estimates derived from the fitted models are summarised in Table A.1, which also details the RMSE for each curve fitted to the data. The generation time at 4°C and $a_w 0.992$ predicted by the Pseudomonas Predictor model is 5.8 hours (95% CI: 5.5 – 6.1 hours) while that predicted at 8°C and $a_w 0.992$ is 3.23 hours (95% CI: 2.9 – 3.6 hours).

Growth conditions	Figure	Gen. Time (h)	Lag Time (h)	RMSE
4°C; low starting level	1	9.5	42.9	23.3
4°C; high starting level	2	6.6	19.5	7.71
8°C; low starting level 8°C; low starting level –	3a	1.7	72.9	43.6
initially constrained fitting	3b	3.1	65.7	47.4
8°C; high starting level	4	3.8	14.7	9.8

Table A. 1 Growth rate and lag time estimates for Pseudomonas growth on lamb carcasees

Discussion

The predicted generation times from the Pseudomonas Predictor model are shorter than what was observed in the experiments conducted and, for three of the four datasets; the observed rates were also outside of the 95% confidence intervals for predicted generation time.

It should be remembered, however, that there are other constraints on the growth of pseudomonads on carcases that are not considered in the model, i.e. pH and lactic acid concentration. These factors are not considered in the Pseudomonas Predictor model. To estimate the potential magnitude of the effect of these factors, we can consider their effects on growth of *E. coli*. Using the *E. coli* model employed in the Refrigeration Index, reducing pH from 7 to 5.8, and increasing lactate from 0 to 80mM, results in an increase in generation time by ~20%. If the same 'correction' were applied to the predicted Pseudomonas generation times, they predicted generation times at 4°C and 8°C would be 7 and 3.9 hours respectively. Equally, it is not known how well controlled the temperature of storage was. A 0.5° C reduction in temperature would alter the predicted generation times to 6.3 and 3.5 hours at 3.5° C and 7.5° C respectively. Surface drying of the carcase could also be expected but was not included in the calculation. A reduction in the water activity to 0.985 increases the predicted generation times to 6.9 and 3.8 hours at 4° C and 8° C, respectively.

Overall, while the data are not in perfect agreement with the predicted generation times, they are consistent with predictions given the lack of the lack of pH and lactic acid terms in the model and uncertainty in the true value of water activity experienced by the cells. The results suggest, however, that a model for Pseudomonas growth on carcase meat should include terms to account for pH and lactic acid conditions that are sub-optimal for Pseudomonas growth.

References

- Neumeyer, K, Ross, T, Thomson, G. and McMeekin, T.A. (1997). Validation of a model describing the effects of temperature and water activity on the growth of psychrotrophic pseudomonads. *International Journal of Food Microbiology*, **38**: 55 63.
- Neumeyer, K, Ross, T, and McMeekin, T.A. (1997). Development of a predictive model to describe the effects of temperature and water activity on the growth of spoilage pseudomonads. *International Journal of Food Microbiology*, **38**: 45-54.

8.2 Appendix 2: Industry Workshop

The outcomes of this project were communicated to industry via the MINTRAC Quality Assurance Managers Network Meeting, held in Melbourne on Friday 11th April, 2008 at Melbourne University Private, Hawthorn.

These forums are advertised on the MINTRAC website and are held regularly for industry across the country.

These forums provide the mechanism for communicating the project outcomes to the target industry audience. Given the success of the above session, an ideal communication strategy for this project would be to 'roadshow' the project outcomes at other Quality Assurance Managers Network meetings across Australia.

The April meeting programmed several speakers, including Ms Bobbitt, DPI Victoria. Approximately 21 participants joined the meeting and represented industry, AQIS, MLA, MINTRAC and DPI.

The presentation as it was delivered is attached below.

MINTRAC QA manager meeting April 08 presentation



Evaluation of the industry communication

The presentation was structured to give an overview of the outcomes of project A.MFRS.0117 in the context of managing export shipments of chilled red meat. The information was delivered as a PowerPoint presentation after which the audience was given the opportunity to pose questions. At the completion of the presentation, attendees were asked to complete a short evaluation form to assess the suitability of the information delivered and rate the performance of the presenter.

The evaluation consisted of four questions. The first question was included for the attendees to self-assess their general knowledge of the topic prior to the presentation. Attendees were asked to circle their desired response.

Question A). Before this presentation I had a (good / reasonable / limited) knowledge of the subject

The final three questions requested that the attendees rate three aspects of the presentation using a rating system of Excellent, Very Good, Good, Adequate, Poor.

Question B). Please rate each section

- i). Information Supplied
- ii). Suitability of the presentation for the meeting
- iii). Quality of Presentation

In addition to the above questions, participants in the session were also given the opportunity to provide further written comments or recommendations to assist in the improvement of the presentation of the information delivered.

Twelve participant feedback forms were returned. Of those 7 (58%) completed Question A, 12 (100%) completed Question B and 5 (42%) added additional comments.

The following responses were returned;

Question A)

- 1 Participant rated their prior knowledge as 'Good to Reasonable'
- 3 Participants rated their prior knowledge as 'Good'
- 2 Participants rated their prior knowledge as 'Reasonable'
- 1 Participant rated their prior knowledge as 'Limited'

Question B)

The following table shows the number of participants who rated the session for each of the scoring categories:

	Excellent	Very Good	Good	Adequate	Poor	Total Responses
B) i	3	2	7			12
B) ii	1	5	5	1		12
B) iii	2	1	8	0.5	0.5	12

The following graph details each participant's score for the series of questions asked for Question B. Scores ranged from 5, for an 'excellent' response, to 1, for a 'poor' response.



<u>Comments</u>

The following comments were received and the presenter's responses have been included.

"Well explained"

This comment is self explanatory and therefore does not require a response

"Make the writing on slides larger"

This may have pertained to the size of the graphs as they did present small and difficult to read.

"Allow the Presenter a little more time to prepare"

Part of the peril of presenting the work of other researchers – one is never as intimate with the work as the project leader.

"Graphs need to be clearer, have handout/presentation notes to follow with would assist"

Slide or Presentation Handout hard copies can be prepared in future to give to the attendees during the presentation, which also allows them to make notes during the delivery. An alternative is to offer electronic copies via email following the presentation.

"Need to get the level of math and micro right for the audience"

This can be difficult to manage as there is often a vast difference in the educational background of personnel in this industry. Presentations are written using a 'middle ground' approach in the aim of addressing the requirements adequately for all levels. Often, however, there are a small number of participants who find the material presented either too difficult or too easy. This is usually addressed by offering further information above the level delivered to those needing greater detail at a more complex level and conversely giving extra attention, where appropriate, to attend to the needs of those that find the material too difficult.

"The lead up to the model ie. The research protocol was a bit long" This could be shortened in future presentations, though it is important to

context how the model fits into the overall management of markets, beyond the scientific findings. Perhaps a forum 'dedicated' to managing markets would be useful to the industry.

"Need to give an idea of where to from here"

Ian Jensen elaborated on this during question time. The model does require some refinement to validate in red meat, which requires funding, that neither MLA nor DPI Victoria can commit to as yet.

"Need a clear intro on what the speaker is going to tell the audience" Improve the introduction of the material and simplify the contextualisation of the work.

The last four comments were received by one participant, who gave the lowest overall score for the presentation.

Some of the comments given are difficult to respond to as they contain insufficient detail to fully address the participants desired outcome. However, all responses should be considered upon subsequent delivery of the project presentation.

Overall the presentation can be considered to have been appropriate to the needs of the participants as 94% of the responses rated the presentation at a level of 'good' or higher. Of this, 41% rated the presentation as 'very good' to 'excellent'.

Meat Microbiology Presentation Participants Feedback

Name:.....(optional) Workshop Location:.....

To maintain the highest quality of information at future MLA workshops we would appreciate your feedback.

Please complete the following questions and return to the presenter.

"Food Spoilage Predictor" – Validation in red meat Speaker – Ms Joanne Bobbitt

A) Before this presentation I had a (good/reasonable/limited) knowledge of the subject. (Please circle)

B) Please rate each section using the following: (Please tick)

	Excellent	Very Good	Good	Adequate	Poor
Information supplied					
Suitability of presentation for the meeting					
Quality of presentation					

C) Please provide any further comments or recommendations to assist in the improvement of the presentation of this information

THANK YOU FOR YOUR COOPERATION. IT IS GREATLY APPRECIATED WE HOPE YOU ENJOYED THE MEATMICROBIOLOGYPRESENTATION



