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Determination of Microbial Lag Phase as Applied to the Process Hygiene Index

John Mills, Colleen Ross, Amanda Gardner and Sushma Prakash

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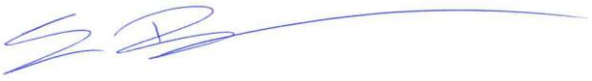
Inquiries or requests to:

John Mills
john.mills@agresearch.co.nz
Food & Bio-based Products Group, AgResearch Ltd
Grasslands Research Centre, Private Bag 11008, Palmerston North, New Zealand



Dr Gale Brightwell
Science Team Leader
Food Assurance Team
Food & Bio-based Products Group

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1. Executive Summary

A series of studies were conducted to determine whether bacteria of sanitary significance in meat production incurred a significant lag phase following attachment to a newly slaughtered piece of meat.

Simulations were constructed with hot-boned striploins from a local export meat premises to study the effect of inoculation onto coupons of muscle and adipose tissue, followed by exposure to a typical cooling curve for four hours.

The bacteria investigated were *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella enterica*. A cocktail of five strains, each isolated from beef and determined to be genetically distinct from each other, was investigated in each case.

A lag time of 30 minutes appears to be defensible based on this work. This may be extendable to 60 minutes if *E. coli* only were to be considered.

2. Background

The Process Hygiene Index (PHI) is currently used by the meat industry as a food safety tool to validate routine chilling processes, or to make decisions about product end use following a non-standard chilling process. The chilling process is considered to be the period from immediately after slaughter and dressing, up until the product reaches and remains below 7°C.

In 2017 a PHI Refresh Project was completed which investigated and evaluated the current Process Hygiene Index used in meat processing plants to ensure the chilling of product post slaughter and dressing to X °C is sufficiently fast to limit the growth of pathogenic bacteria on the meat surface, for which *Escherichia coli* is the established indicator bacterium. This project found that while the current PHI and its customised form was fit for purpose, it may be overly conservative. One factor that may require further consideration is that of lag time, the time from when bacteria are transferred to the carcass surface to when they begin to proliferate on the new matrix (Gill, 1991).

In Australia, a lag phase of growth is included in the Refrigeration Index (RI) for meat that starts as ‘hot’ or ‘warm’, to account for the bacteria adjusting to the new environment of the carcass surface. The lag phase is accounted for by deducting 5 generations from the calculated RI. This lag was based on two studies:

Smith (1985) describes experiments with sheep meat taken direct from the slaughter line, surface inoculated with *E. coli* and then blended. At 40°C, the generation time was 0.3 hours with a lag time of 1.4 hours, resulting in an observed lag period lasting 4.6 times the expected generation time at this temperature. At 35 and 30°C, the lag time was equivalent to 3.2 and 2.9 times the expected generation time.

Ross (1999) conducted a survey of published and private experiments considering lag time before growth on inoculated foods and broths. The distribution of lag times of *E. coli* on foods showed a sharp peak at 3 generation time equivalents, whereas *E. coli* growing in broth was found to show a distribution curve of lag time peaking around 5 generation time equivalents.

This study is intended to provide an in-depth examination of lag times for late exponential phase bacteria inoculated onto beef for the first four hours post slaughter.

3. Methods

This study utilised strains of *Escherichia coli*, *Staphylococcus aureus* and *Salmonella enterica* serovars that had been isolated from beef carcasses. Five strains were selected for each species (refer 4.1 for designations of strains selected), which were confirmed as genetically distinct either by ERIC-PCR fingerprinting (for *E. coli* and *S. aureus*), or serotyping (for *S. enterica*).

The basic protocol was adapted from Gill et al., (2001), and unlike many previous growth experiments was based on growth of the target bacteria on actual meat tissue rather than *in-vitro* studies in broth. Adipose and muscle tissue are considered separately as these tissue types are both present in beef carcasses and primal cuts and have different substrate availabilities and pH characteristics, particularly as the tissues age post slaughter.

3.1 Collection and verification of target bacteria

- *E. coli* were isolated by culturing the sample homogenates from sponge swab samples that had been taken from beef sides at an export meat premises onto *E. coli* Petrifilm™ plates (3M, Maplewood USA) (APHA, 2001a). Typical colonies were subcultured for purity onto MacConkey agar (Fort Richard, Auckland).
- Strains of *S. aureus* of beef origin had been isolated previously for a historical project examining relationships between beef and human strains. Strains were revived from frozen glycerol stock cultures and resuscitated onto sheep blood agar (Fort Richard, Auckland). Five strains were selected for verification.
- Four different serotypes of *S. enterica* isolated from beef samples, Bovismorbificans 18ER3848, Enteritidis phage type 11 18ER4603, Stanley 18ER3501 and Typhimurium phage type 101 18ER4853 were obtained from ESR, and selected alongside a previously characterised strain of *S. Brandenburg* isolated from South Island beef by AgResearch.
- Sanger sequencing of the 16S rRNA gene was undertaken to confirm the identity of *E. coli* and *S. aureus* used in this study, using the protocol described by Brightwell et al. (2006). ERIC-PCR was used to demonstrate that each individual *E. coli* and *S. aureus* strain was associated with a distinct profile using gel electrophoresis (Rapp et al., 2018). Five distinct strains of *E. coli* and *S. aureus* were then selected for use in the lag phase studies.
- The growth potential of each selected strain was determined by inoculating each organism into 10 mL of sterile tryptic soy broth (TSB, Fort Richard Auckland) and incubating for 18 hours at 35°C. Each strain was then subcultured (100 µL) into fresh TSB (10 mL) and incubated again for 18 hours [1] at 35°C. A tenfold dilution series (to $1:10^{10}$) was then prepared for each individual culture by removing 100 µL growth and spread plating onto plate count agar plates (PCA, Fort Richard, Auckland). The plates were incubated 18-22 hours at 35°C, then plates with 20-200 colonies were counted. From these counts the dilution of enrichment culture required to obtain equal portions of each strain for inclusion in a cocktail for inoculation was calculated.
- The inoculum level selected for each group of bacteria was based on practical limitations of the methodologies for detecting countable bacteria from meat surfaces. Factors considered included the sample dilutions necessary to prepare a liquid sample suspension, the amount of the suspension that could be pipetted onto the detection plate, and the relative abundance of meat particles present in higher sample dilutions and how they might interfere with the detection system. These factors resulted in a final sample inoculum being used between 10^3 to 10^4 cfu of cocktail being inoculated per 100 µL of suspension.

[1] For the bacteria selected, 18 hours represents late exponential to early stationary phase.

3.2 Lag phase experiments

Three trial runs of the following protocol were conducted for each of the bacterial species under trial (*E. coli*, *S. aureus* & *S. enterica*, nine runs in total):

- Beef striploins were obtained hot-boned from a local export meat production premises. Estimated elapsed time from slaughter to inoculation was 70-100 minutes. On arrival coupons were held at 37°C for 30 minutes to equilibrate to body temperature.
- Striploin (longissimus dorsi) is comprised of a flat muscle layer overlaid with a membrane, above which are one or two adipose layers. The adipose and muscle layers were separated. Adipose tissue was typically around 10 mm thick; coupons were cut from this of approximately 8cm² area on the upper surface. Muscle tissue was first cut longitudinally into steaks of approximately 10mm thickness, then an excision tool was used to cut coupons of upper surface area 8cm² from this.
- Sets of seven replicate coupons for adipose and muscle tissue were transferred into high wall 200 mm wide sterile Petri dishes, in such a way that each coupon was physically separated (figures 1 and 2). A separate dish was prepared for each time point (0-240 minutes, at 30-minute intervals), for both adipose and muscle tissue.

Figure 1 – seven replicates of adipose tissue

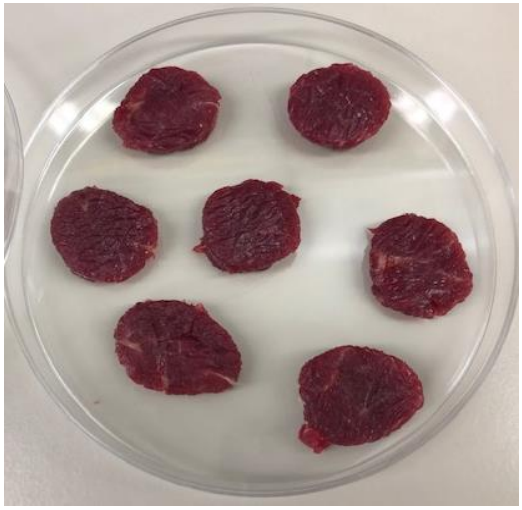

Figure 2 – seven replicates of muscle tissue

- The lid of each dish was removed and the surface of each coupon was inoculated with 100 µL of the appropriate bacterial cocktail. The lid was replaced and the dish held until all dishes had been inoculated. Uninoculated control sets were also prepared.
- Time 0 and control dishes were sampled immediately. Dishes for time +30minutes to time + 240 minutes were transferred to an environment chamber and subjected to the cooling curve in figure 3. Samples were withdrawn for analysis at each time point. The figure shows the modelled cooling curve and logger data from the chamber during trial run to match the curve. This curve is based on Kemp (2016) and source data provided by MPI (Langdon, 2019):

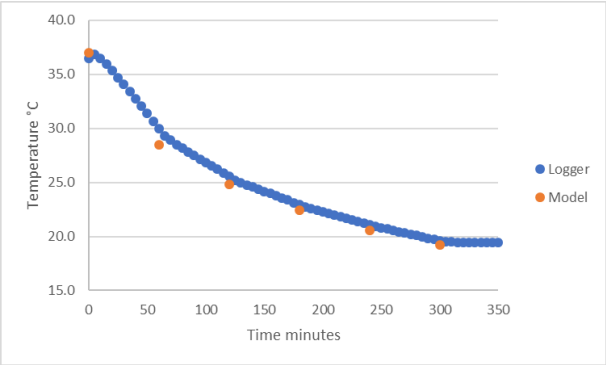

Figure 3 - temperature curve for beef cooling

- The seven coupons from each set were analysed separately as follows:
  - Each coupon was removed from the dish and placed in a sterile Whirlpak bag (Nasco, Wisconsin, USA) with 40 mL of maximum recovery diluent (MRD, Fort Richard, Auckland) and stomached for 2 minutes high speed in a Seward 400 stomacher (Seward, UK). Tenfold dilutions were made in MRD.
  - For samples inoculated with *E. coli*, 1 mL of each dilution was plated onto *E. coli* Petrifilm™. Plates were incubated at 35°C and blue colonies associated with gas bubbles counted on plates containing 20-200 colonies.
  - For samples inoculated with *S. aureus*, 1 mL of each dilution was plated onto Staphylococci Petrifilm™. Plates (3M, Maplewood, USA) according to manufacturer's instructions and were incubated at 35°C for 22 hours. Red colonies were counted on plates containing 20-200 colonies. Confirmation as *S. aureus* was performed on the first set of samples using the Petrifilm DNAase insert in accordance with manufacturer's instructions, with positive colonies producing a pink halo.
  - For samples inoculated with *Salmonella* spp., 0.1 mL of each dilution was plated onto xylose lysine desoxycholate medium (XLD, Fort Richard Auckland) and spread plated to produce countable colonies. Plates were incubated at 35°C and red colonies with a black centre were counted on plates containing 20-200 colonies (AgResearch 2019). Typical colonies were confirmed using the Oxoid Salmonella test kit in accordance with manufacturer's instructions (DR1108A, Oxoid, Basingstoke, UK).

3.3 Statistical analysis

For each experimental series, it was determined that seven replicates were required per time point. This was based on a power analysis of 80% probability of showing a 5% difference where a 0.5 $\log_{10}$ cfu.cm^{-2} is considered significant and a range of data of up to 3 logs is expected (Pearson & Hartley, 1970).

Determination of the actual lag time was calculated by fitting the PHI growth model onto the growth curves obtained from these experiments. The time before the logarithmic phase of the curve commencing was considered as the lag phase.

4. Results and Discussion

4.1 Bacteria inoculated

The following strains were isolated from beef samples, identified as *E. coli* by Sanger sequencing of the 16S rRNA gene and found to be distinguishable from each other by ERIC-PCR:

C16A, C17A, C18A, C24A and C25A.

The different strains were combined together at 10^{-4} dilution to provide the required inoculum.

S. aureus strains were obtained from a previous study on beef. The following strains were confirmed as S. aureus by Sanger sequencing of the 16S rRNA gene and found to be distinguishable from each other by ERIC-PCR:

T11B, T11F, T12C, T32F, T44G.

The same inoculum level was used initially, however meat fibres in the suspension made the undiluted suspension difficult to count. The inoculum was therefore raised to provide a count of 10^4 cfu/sample at time 0.

The different serotypes of *Salmonella* enterica described previously were also inoculated to provide a count of 10^4 cfu/sample at time 0.

4.2 Growth results

The bacterial viable count results obtained after incubation of adipose tissue with all three inoculated species consistently revealed a short lag phase followed by exponential growth. The bacterial viable count results from muscle tissue were more variable, with some results indicating a similar curve to adipose tissue and other data indicating that exponential growth was not achieved until the end of the 240 minute trial period. This variability of growth curves obtained from respective samples is illustrated below with experiments involving *S. enterica* (figures 4 and 5).

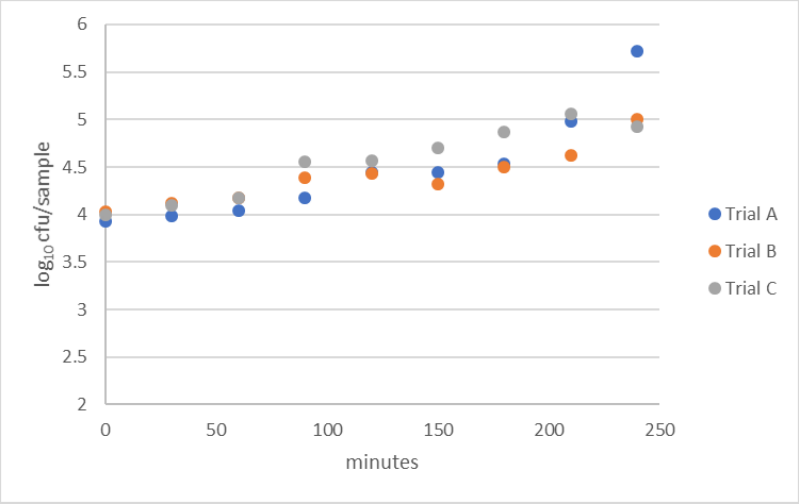

Figure 4 - growth curve for adipose tissue inoculated with S. enterica

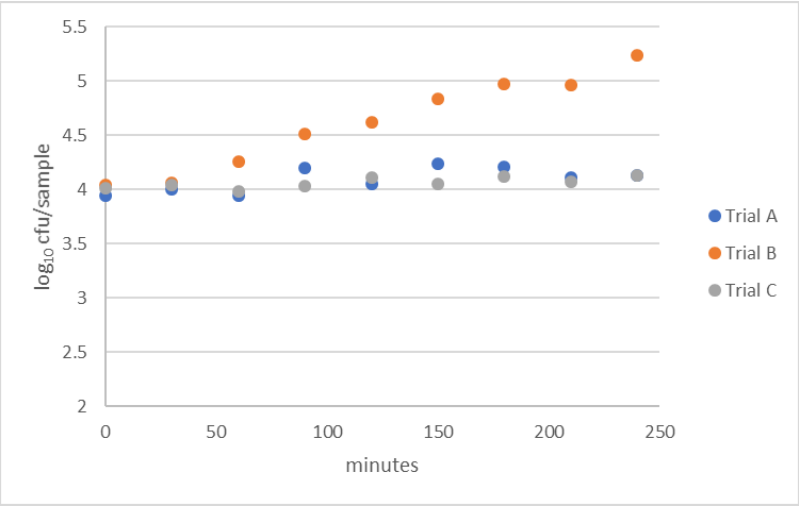

Figure 5 - growth curve of muscle tissue inoculated with S. enterica

pH measurement data obtained as samples were inoculated (for *Salmonella*, table 1) led to a hypothesis that muscle samples with a pH of ≤6.0 may be less likely to support bacterial growth, as these tissues with a lower pH may contain less stored glucose. There is however insufficient data to test this hypothesis from this study. Following discussions with the modelling team at ESR, it was decided that the growth data from adipose tissue showed a shorter lag phase, and so these data were selected for further analysis to fit the modelled growth curves to calculate the lag time on this tissue.

Table 1 - pH of striploin inoculated with Salmonella spp.

Sample date	Adipose pH	Muscle pH
13/8/19	6.4 ± 0.2	6.0 ± 0.25
20/8/19	6.5 ± 0.25	6.2 ± 0.1
22/8/19	6.4 ± 0.15	5.9 ±0.3

The results of the three replicate experiments for each species are displayed graphically in figures 6-8 (*E. coli* inoculated), 9-11 (*S. aureus* inoculated) and 12-14 (*S. enterica* inoculated). In these figures, the model PHI curve has been superimposed over the actual growth curve, with the logarithmic phase commencing at 30-minute intervals from time 0 (undivided line time 0, dotted line, time +30 minutes, dashed line time +60 minutes).

These results indicate that a 60-minute lag time may be satisfactory for *E. coli*, but 30 minutes appears more defensible for *S. aureus* and *S. enterica*.

The complete sets of results are provided in appendices 1-3.

4.2.1 *Escherichia coli*

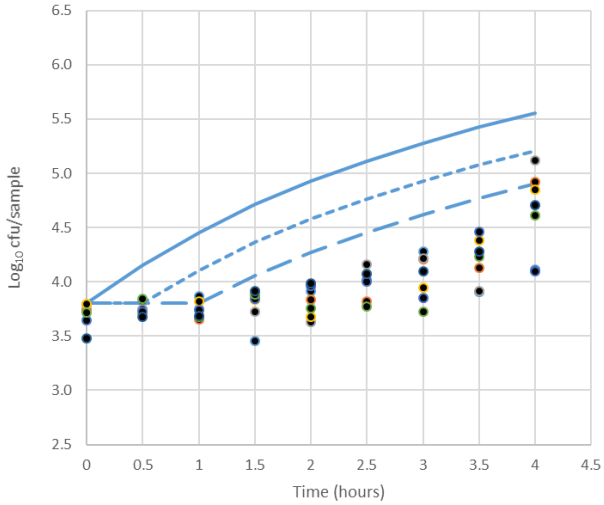

Figure 6 - Adipose tissue inoculated with E. coli, run 1

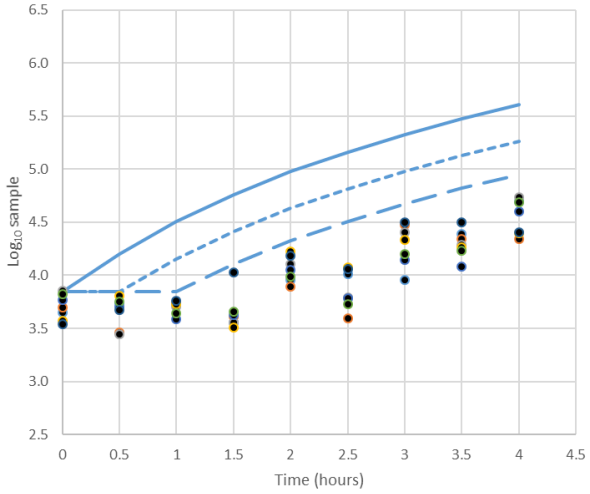

Figure 7 - Adipose tissue inoculated with E. coli, run 2

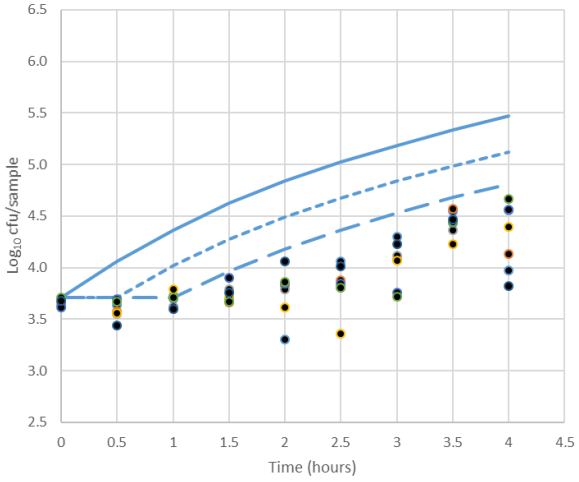

Figure 8 - Adipose tissue inoculated with E. coli, run 3

4.2.2 *Staphylococcus aureus*

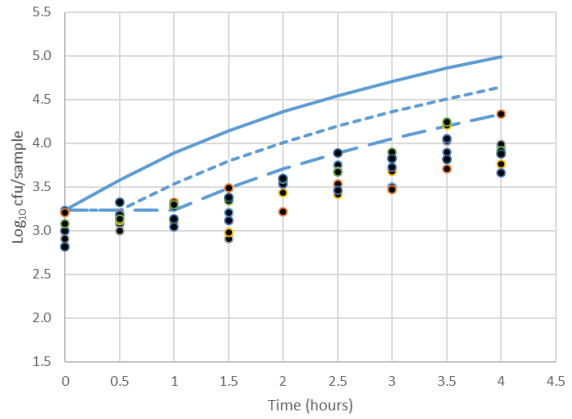

Figure 9 - Adipose tissue inoculated with S. aureus, run 1

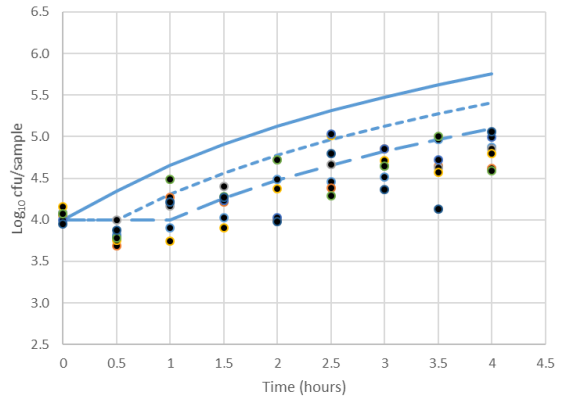

Figure 10 - Adipose tissue inoculated with S. aureus, run 2

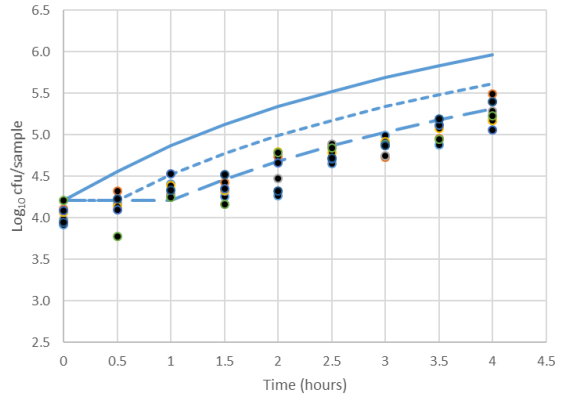

Figure 11 - Adipose tissue inoculated with S. aureus, run 3

4.2.3 *Salmonella enterica*

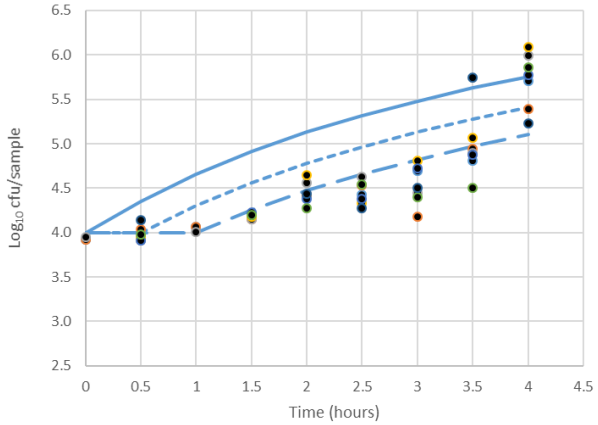

Figure 12 - Adipose tissue inoculated with S. enterica, run 1

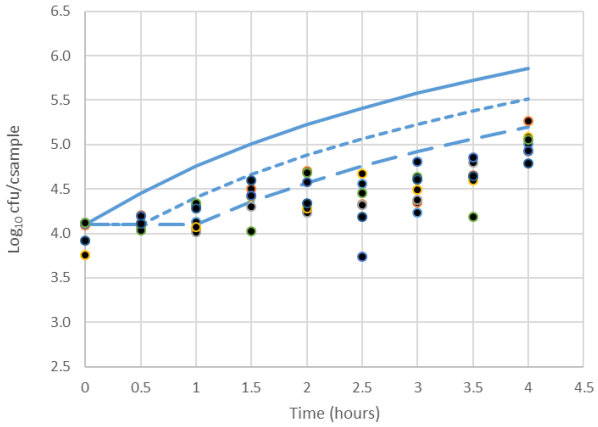

Figure 13 - Adipose tissue inoculated with S. enterica, run 2

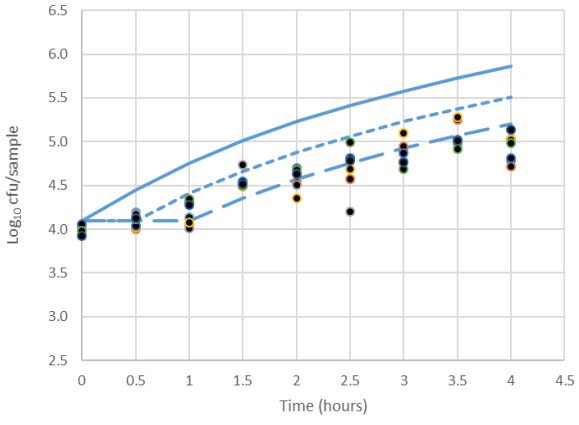

Figure 14 - Adipose tissue inoculated with S. enterica, run 3

5. Recommendations

A lag time of 30 minutes appears to be defensible based on this work. This may be extendable to 60 minutes if *E. coli* only were to be considered.

The extended lag time observed during some runs is worthy of further investigation. An initial hypothesis was that the pH of the meat matrix may have some impact, but the internal muscle variation was such that this cannot be determined from existing data. It should also be noted that these results are based on bacteria at a particular phase of growth (18 hours) and the lag may differ with bacteria in early lag or late stationary phases.

6. Acknowledgements

We wish to acknowledge Eden Esteves for collecting the beef samples from which *E. coli* strains were isolated, and Faith Palevich for performing the original isolation work. Jackie Boerema led the project on beef from which the taphylococci strains were obtained. Our thanks also to Jackie Wright (ESR) for supplying the *Salmonella* isolates. Particular thanks to ANZCO for supplying the beef striploins.

Thanks to Harold Henderson for the initial power analysis, and Maryann Staincliffe and Martin Upsdell for statistical analysis. Thanks also to Beverley Horn (ESR) for fitting the PHI model into the final graphical figures.

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8. Appendices

8.1 *E. coli* results

Run 1

Adipose

	A0	A1	A2	A3	A4	A5	A6	A7	A8
	3.740	3.823	3.863	3.455	3.911	4.017	4.278	3.906	4.114
	3.740	3.736	3.653	3.875	3.839	3.816	4.204	4.130	4.919
	3.789	3.744	3.748	3.720	3.628	4.163	4.212	3.911	5.122
	3.799	3.699	3.820	3.839	3.677	4.072	3.942	4.380	4.851
	3.643	3.720	3.740	3.842	3.959	4.000	3.854	4.462	4.097
	3.716	3.842	3.677	3.881	3.756	3.771	3.724	4.230	4.607
	3.477	3.677	3.686	3.914	3.987	4.074	4.100	4.279	4.708
Mean	3.701	3.749	3.741	3.789	3.822	3.988	4.045	4.186	4.631
SD	0.111	0.062	0.078	0.160	0.140	0.143	0.208	0.217	0.394

Muscle

	M0	M1	M2	M3	M4	M5	M6	M7	M8
	3.756	3.813	3.916	3.949	4.158	4.330	4.491	5.328	5.107
	3.771	3.653	3.878	4.031	4.238	4.281	4.513	5.203	5.377
	3.740	3.775	3.820	4.023	4.154	4.410	4.554	5.172	5.354
	3.748	3.833	3.789	4.004	4.125	4.319	4.494	5.412	5.314
	3.708	3.732	3.740	3.914	4.124	4.326	4.524	4.989	4.980
	3.789	3.648	3.872	4.117	4.175	4.288	4.494	5.314	4.968
	3.748	3.792	3.919	4.002	4.119	4.346	4.505	5.322	5.152
Mean	3.751	3.749	3.848	4.006	4.156	4.329	4.511	5.249	5.179
SD	0.025	0.074	0.067	0.065	0.042	0.043	0.022	0.140	0.172

	Minutes
T0	0
T1	30
T2	60
T3	90
T4	120
T5	150
T6	180
T7	210
T8	240

Run 2

Adipose

	A0	A1	A2	A3	A4	A5	A6	A7	A8
	3.648	3.736	3.736	3.556	3.957	4.015	3.954	4.389	4.695
	3.695	3.462	3.736	3.607	3.892	3.591	4.477	4.342	4.342
	3.851	3.447	3.703	3.556	4.107	3.792	4.407	4.290	4.736
	3.568	3.806	3.732	3.505	4.221	4.076	4.332	4.267	4.398
	3.771	3.708	3.585	3.623	4.049	3.782	4.146	4.079	4.602
	3.820	3.752	3.643	3.658	3.985	3.732	4.204	4.230	4.690
	3.538	3.677	3.760	4.029	4.188	4.063	4.498	4.498	4.407
Mean	3.699	3.655	3.700	3.648	4.057	3.864	4.288	4.300	4.553
SD	0.121	0.143	0.063	0.176	0.122	0.187	0.198	0.132	0.166

Muscle

	M0	M1	M2	M3	M4	M5	M6	M7	M8
	3.672	3.643	3.916	3.993	4.079	4.380	4.519	4.708	4.942
	3.760	3.752	3.878	3.980	4.217	4.290	4.352	4.667	4.878
	3.732	3.724	3.820	3.966	4.114	4.371	4.544	4.686	4.934
	3.686	3.672	3.789	3.949	4.061	4.362	4.550	4.628	5.009
	3.720	3.732	3.740	3.954	4.146	4.352	4.519	4.556	4.934
	3.613	3.782	3.872	3.900	4.146	4.243	4.613	4.672	4.748
	3.681	3.775	3.919	3.872	4.041	4.362	4.658	4.525	4.519
Mean	3.695	3.726	3.848	3.945	4.115	4.337	4.536	4.635	4.852
SD	0.048	0.051	0.067	0.044	0.061	0.051	0.096	0.069	0.168

Run 3

Adipose

	A0	A1	A2	A3	A4	A5	A6	A7	A8
	3.638	3.638	3.728	3.690	3.301	4.057	4.301	4.544	3.971
	3.667	3.574	3.712	3.658	3.820	3.878	4.079	4.574	4.130
	3.699	3.550	3.618	3.785	3.789	3.806	4.119	4.362	4.556
	3.695	3.556	3.789	3.720	3.613	3.362	4.068	4.230	4.398
	3.613	3.695	3.699	3.898	3.842	3.848	3.756	4.431	4.562
	3.712	3.672	3.712	3.672	3.863	3.806	3.716	4.439	4.667
	3.677	3.439	3.597	3.760	4.063	4.015	4.230	4.470	3.823
Mean	3.672	3.589	3.693	3.740	3.756	3.825	4.039	4.436	4.301
SD	0.035	0.087	0.066	0.083	0.240	0.227	0.223	0.115	0.328

Muscle

	M0	M1	M2	M3	M4	M5	M6	M7	M8
	3.699	3.628	3.597	3.686	3.538	3.568	3.607	3.854	3.597
	3.602	3.602	3.681	3.663	3.623	3.954	3.574	3.810	3.643
	3.716	3.602	3.574	3.525	3.699	3.628	3.677	3.911	3.415
	3.544	3.699	3.628	3.653	3.724	3.585	3.663	3.562	3.839
	3.677	3.643	3.602	3.716	3.613	3.491	3.823	3.829	4.116
	3.740	3.663	3.574	3.771	3.628	3.712	3.484	3.525	3.799
	3.663	3.708	3.602	3.602	3.653	3.789	3.792	3.681	4.006
Mean	3.663	3.649	3.608	3.659	3.640	3.675	3.660	3.739	3.774
SD	0.068	0.043	0.037	0.079	0.061	0.157	0.119	0.151	0.243

8.2 *S. aureus* results

Run 1

Adipose									
	A0	**A1**	**A2**	**A3**	**A4**	**A5**	**A6**	**A7**	**A8**
	3.230	3.079	3.267	3.204	3.544	3.748	3.498	3.900	3.971
	3.204	3.190	3.322	3.491	3.217	3.531	3.470	3.703	4.332
	2.903	3.000	3.114	2.903	3.591	3.681	3.833	4.025	3.987
	3.000	3.114	3.097	2.978	3.431	3.415	3.677	4.207	3.760
	3.000	3.176	3.041	3.114	3.531	3.462	3.720	4.055	3.663
	3.079	3.130	3.301	3.342	3.585	3.672	3.898	4.246	3.916
	2.813	3.322	3.130	3.380	3.597	3.889	3.820	3.813	3.875
Mean	3.033	3.145	3.182	3.202	3.500	3.629	3.702	3.993	3.929
SD	0.152	0.101	0.112	0.217	0.137	0.168	0.166	0.200	0.212

Muscle									
	M0	**M1**	**M2**	**M3**	**M4**	**M5**	**M6**	**M7**	**M8**
	3.097	3.190	3.176	3.204	3.279	3.079	3.301	3.000	3.633
	3.161	3.407	3.217	3.130	3.114	3.484	3.380	3.550	3.332
	3.146	3.130	3.176	3.290	2.978	3.267	3.423	3.176	3.439
	3.079	3.204	3.130	3.362	3.204	3.279	3.531	3.352	3.423
	3.161	3.301	3.114	3.322	3.114	3.312	3.312	3.312	3.290
	3.243	3.217	3.190	3.114	3.176	3.332	3.371	3.484	3.398
	3.190	3.312	3.342	3.161	3.217	3.279	3.491	3.389	3.531
Mean	3.154	3.252	3.192	3.226	3.155	3.290	3.401	3.323	3.435
SD	0.055	0.093	0.075	0.098	0.097	0.119	0.087	0.187	0.117

	Minutes
T0	0
T1	30
T2	60
T3	90
T4	120
T5	150
T6	180
T7	210
T8	240

Run 2

Adipose									
	A0	**A1**	**A2**	**A3**	**A4**	**A5**	**A6**	**A7**	**A8**
	4.079	3.816	3.903	4.021	4.484	4.462	4.519	4.968	4.875
	4.053	3.690	4.267	4.217	4.000	4.380	4.854	4.648	4.618
	4.079	4.000	4.166	4.398	4.724	4.667	4.648	4.628	4.854
	4.155	3.748	3.740	3.903	4.371	5.013	4.708	4.568	4.796
	3.998	3.789	4.217	4.230	4.021	5.033	4.851	4.720	4.993
	4.068	3.785	4.484	4.279	4.720	4.290	4.648	5.000	4.591
	3.954	3.878	4.217	4.267	3.978	4.796	4.362	4.130	5.064
Mean	4.055	3.815	4.142	4.188	4.328	4.663	4.656	4.666	4.827
SD	0.064	0.100	0.246	0.168	0.332	0.299	0.176	0.290	0.177

Muscle									
	M0	**M1**	**M2**	**M3**	**M4**	**M5**	**M6**	**M7**	**M8**
	3.875	4.114	3.875	4.161	4.230	4.290	4.290	4.512	4.568
	4.217	4.114	4.041	3.954	3.978	4.097	4.279	4.512	4.607
	4.243	4.190	3.845	3.978	4.079	4.079	4.362	4.491	4.585
	4.130	3.954	4.021	4.161	4.146	4.176	4.290	4.371	4.716
	4.114	4.079	3.740	4.204	4.161	4.000	4.371	4.230	4.439
	4.097	4.097	3.954	4.000	4.000	4.176	4.301	4.512	4.574
	4.130	4.114	4.041	4.061	3.954	4.097	4.312	4.371	4.591
Mean	4.115	4.095	3.931	4.074	4.078	4.131	4.315	4.429	4.583
SD	0.119	0.071	0.115	0.101	0.105	0.093	0.037	0.108	0.081

Run 3

Adipose									
	A0	**A1**	**A2**	**A3**	**A4**	**A5**	**A6**	**A7**	**A8**
	3.916	4.161	4.407	4.255	4.267	4.658	4.998	4.878	5.176
	4.104	4.322	4.279	4.423	4.736	4.878	4.724	4.954	5.498
	3.982	4.176	4.279	4.322	4.470	4.889	4.752	5.116	5.290
	4.068	4.130	4.389	4.332	4.792	4.785	4.922	5.079	5.176
	4.088	4.097	4.531	4.352	4.667	4.712	4.892	5.116	5.061
	4.214	3.778	4.243	4.161	4.782	4.845	4.884	4.942	5.230
	3.949	4.230	4.332	4.519	4.322	4.716	4.875	5.190	5.398
Mean	4.046	4.128	4.351	4.338	4.577	4.783	4.864	5.039	5.261
SD	0.103	0.171	0.100	0.114	0.221	0.091	0.095	0.115	0.148

Muscle									
	M0	**M1**	**M2**	**M3**	**M4**	**M5**	**M6**	**M7**	**M8**
	4.279	4.097	4.217	3.875	4.097	4.290	4.146	4.342	4.362
	4.290	3.875	4.146	4.130	4.079	4.230	4.161	4.267	4.322
	4.230	3.875	3.875	4.061	4.398	4.061	4.061	4.398	4.371
	4.230	4.061	4.041	4.204	4.255	4.267	4.243	4.255	4.000
	4.217	3.903	4.146	4.114	4.190	4.230	4.021	4.332	4.628
	4.255	4.114	4.176	4.000	4.130	4.312	4.230	4.279	4.243
	4.097	4.021	4.176	4.041	4.301	4.255	4.322	4.061	4.312
Mean	4.228	3.992	4.111	4.061	4.207	4.235	4.169	4.276	4.320
SD	0.064	0.105	0.117	0.105	0.117	0.082	0.106	0.107	0.186

8.3 Salmonella results

Run 1																					
Adipose											*Muscle*										
	A0	A1	A2	A3	A4	A5	A6	A7	A8			M0	M1	M2	M3	M4	M5	M6	M7	M8			Minutes
	3.916	3.952	4.064	4.173	4.398	4.431	4.690	4.806	5.708			3.975	3.860	3.816	4.447	4.290	4.079	4.204	4.362	3.845		T0	0
	3.924	4.037	4.061	4.146	4.431	4.531	4.176	4.944	5.389			3.968	4.027	4.415	3.924	4.161	4.667	3.954	4.648	3.813		T1	30
	3.954	3.911	4.004	4.149	4.556	4.628	4.447	4.895	5.991			3.914	3.929	3.869	4.021	3.903	4.061	4.021	4.021	4.556		T2	60
		3.940		4.179	4.643	4.332	4.810	5.063	6.088			4.027	3.892	3.919	4.322	3.978	4.161	4.079	3.699	4.658		T3	90
		3.911		4.230	4.380	4.380	4.720	4.875	5.775			3.975	4.290	3.884	4.097	3.845	4.097	4.556	3.903	4.041		T4	120
		3.978		4.193	4.279	4.538	4.398	4.505	5.863			3.810	4.041	3.728	4.580	4.267	4.079	3.929	4.021	4.114		T5	150
		4.145			4.439	4.279	4.505	5.748	5.230				3.973	3.944	3.982	3.903	4.525	4.724	4.114	3.875		T6	180
Mean	3.932	3.982	4.043	4.179	4.447	4.446	4.535	4.977	5.721		Mean	3.945	4.002	3.939	4.196	4.050	4.239	4.210	4.110	4.129		T7	210
SD	0.020	0.084	0.034	0.031	0.120	0.125	0.220	0.381	0.311		SD	0.076	0.143	0.221	0.254	0.186	0.250	0.311	0.311	0.345		T8	240

Run 2																					
Adipose											*Muscle*										
	A0	A1	A2	A3	A4	A5	A6	A7	A8			M0	M1	M2	M3	M4	M5	M6	M7	M8			
	4.121	4.076	4.127	4.423	4.255	4.556	4.230	4.623	5.006			4.031	4.064	4.201	4.322	4.648	4.964	5.169	5.169	5.146			
	4.092	4.203	4.051	4.505	4.703	4.332	4.352	4.653	5.268			4.041	4.072	4.172	4.352	4.505	4.957	4.957	5.027	5.477			
	4.112	4.116	4.013	4.301	4.230	4.322	4.380	4.796	4.922			4.043	3.998	4.276	4.290	4.690	4.906	5.033	4.860	5.041			
	3.752	4.095	4.076	4.415	4.279	4.672	4.491	4.597	5.086			4.064	4.081	4.160	4.623	4.531	4.623	4.903	4.875	5.312			
	4.111	4.200	4.317	4.423	4.574	3.740	4.810	4.854	4.932			4.047	4.085	4.505	4.653	4.736	4.892	4.816	5.166	5.176			
	4.119	4.037	4.341	4.021	4.681	4.455	4.638	4.190	5.051			4.017	4.019	4.319	4.703	4.505	4.740	4.919	4.728	5.243			
	3.919	4.111	4.278	4.597	4.342	4.190	4.607	4.643	4.785			4.045	4.099	4.193	4.607	4.703	4.763	4.991	4.898	5.255			
Mean	4.032	4.120	4.172	4.384	4.438	4.324	4.501	4.622	5.007		Mean	4.041	4.060	4.261	4.507	4.617	4.835	4.970	4.960	5.236			
SD	0.143	0.062	0.137	0.184	0.208	0.303	0.198	0.213	0.152		SD	0.015	0.037	0.122	0.177	0.100	0.128	0.112	0.166	0.138			

Run 3																					
Adipose											*Muscle*										
	A0	A1	A2	A3	A4	A5	A6	A7	A8			M0	M1	M2	M3	M4	M5	M6	M7	M8			
	3.996	4.192	4.134	4.544	4.525	4.816	4.916	4.975	4.785			4.076	4.102	3.914	3.919	4.290	4.097	3.903	4.470	4.512			
	4.029	4.002	4.051	4.531	4.538	4.568	4.944	5.253	4.712			3.978	4.009	3.980	4.057	3.954	3.740	4.290	3.845	4.114			
	4.033	4.167	4.013	4.732	4.505	4.204	4.771	4.980	5.031			4.023	4.041	4.041	4.047	4.061	4.041	4.021	4.000	4.312			
	3.987	4.021	4.076	4.484	4.352	4.690	5.100	5.276	5.019			4.037	4.029	4.009	4.112	4.000	4.161	4.114	3.740	3.813			
	4.053	4.039	4.317	4.544	4.699	4.816	4.866	5.021	4.816			4.015	4.021	3.998	4.039	4.079	4.130	4.176	4.255	4.000			
	3.980	4.117	4.341	4.498	4.677	4.998	4.690	4.916	4.980			3.964	4.135	4.025	3.959	4.114	4.279	4.097	3.929	4.079			
	3.922	4.129	4.278	4.519	4.633	4.782	4.767	5.015	5.132			3.987	3.952	3.927	4.086	4.255	3.875	4.255	4.279	4.097			
Mean	4.000	4.095	4.173	4.550	4.561	4.696	4.865	5.062	4.925		Mean	4.011	4.041	3.985	4.031	4.108	4.046	4.122	4.074	4.132			
SD	0.044	0.075	0.136	0.083	0.120	0.254	0.137	0.142	0.154		SD	0.039	0.061	0.048	0.069	0.125	0.183	0.134	0.265	0.224			