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Executive Summary

There are perceptions that chickens raised on multi-batch litter may be exposed to higher levels of food-borne pathogen loads (such as *Campylobacter* and *Salmonella*) than chickens raised on freshly placed bedding material (i.e. single use litter). These perceptions also contribute to concerns that such increased levels of pathogens would thus increase the load of food-safety pathogens (*Salmonella* and *Campylobacter*) via chicken meat across the food process chain. Such perceptions have an impact not only on the producers who currently re-use litter but also impact on the environment in terms of disposal of litter, due to the possible re-entry of such pathogens to the food chain via the environment. Thus, there are increasing levels of restrictions on the approved end-uses of chicken litter which is destined for agricultural use. In contrast a situation where 100% of meat chickens are raised on fresh bedding material would not only greatly increase the demand for new bedding material but also contribute to an increase in demand for the already decreasing resource from an environmental and economic point of view. Thus in a climate where litter re-use already occurs, it is vital that a scientific basis for what occurs during re-use is established for the benefit of all concerned. Such information will not only benefit the producers but also generate previously unavailable Australian data on litter re-use and the fate of the key food-borne pathogens such as *Salmonella* and *Campylobacter* within a re-use litter scenario.

Thus the objectives of this study can be broadly categorised as follows:-

- Evaluate current practices adopted (e.g. litter pile-up) prior to re-use of litter for subsequent chicken cycles
- To establish pathogen die-off that occurs during currently adopted methods of in-shed treatment of litter
- To establish simple physical parameters to monitor this pathogen reduction and create an understanding of such reduction strategies to aid in-shed management of re-use litter
- To carry out studies to assess the potential of the re-used litter (once spread) to support pathogens during a typical chicken production cycle.
- To provide background data for the development of a simple code of practice for an in-shed litter pile-up process

The project consisted of extensive on-farm studies that were supported by laboratory studies. In both studies, the pile-up and the chicken production cycle, the presence and levels of food-safety pathogens (*Campylobacter* and *Salmonella*) as well as other key target bacteria (*Bacillus* spp, *Clostridium perfringens* and *Escherichia coli*) were evaluated at different stages of the process to evaluate the die-off potential of these organisms. A range of physical parameters i.e. temperature, pH, water activity and moisture content, were also recorded simultaneously during the testing for these organisms.

To achieve the above outcomes, two farms that had a long history of litter re-use were selected. The in-shed litter treatment process was followed on both farms, beginning after a full cleanout. Two litter pile-up processes were followed sequentially on the first farm and three of the five litter push-up processes were followed through on the second farm.

These studies were designed in a manner so that litter was tested prior to push-up, during the push-up process and after the litter was spread in the grow-out end ready for chickens. *Campylobacter* was present on all occasions at high levels (10^4 to 10^6/g) in the litter (at the latter stages of the chicken cycle) before the piling process while *Salmonella* was present on most occasions before the piling process but at lower levels (10^3 to 10^4/g). *Salmonella* and *Campylobacter* showed a decrease in level through the pile-up process with none of these organisms being present on days 4-6, the final days of the pile. In fact *Campylobacter*

demonstrated a 6 log reduction. *E. coli* (an indicator organism), however, was present during the last day of the pile (during both cycles) on the first farm but was not detected on the second farm. This can possibly be linked to the fact that these particular piles had a generally lower temperature profile than that other piles examined in the study. Both *Clostridium perfringens* and *Bacillus*, spore forming organisms, were not impacted by the pile-up process. Hence all outcomes from the pile-up process suggest that this process has the potential to deal with the key pathogens (*Salmonella* and *Campylobacter*) within 4-6 days of pile-up. Thus these pile studies confirmed that the current practices on both farms achieved effective pathogen destruction. It is vital that these outcomes need to be supported by good in-shed management processes.

Based on some of the outcomes of the study it was noted that temperature alone did not contribute to pathogen die-off due to the fact, even in the presence of temperature variations, pathogen die-off was achieved. The other factors contributing to pathogen die-off were intrinsic litter parameters such as pH and water activities (available water for microbial growth). Whilst there were temperature variations across the pile locations, both water activity and pH did not show such marked variations. For example, while certain pile locations may not reach the key temperatures for inactivation a suitable pH can still result in high ammonia generation which will have a bacteriocidal effect or the prevailing water activities, especially in spread litter (prior to chickens) can act inhibitory for pathogen support.

These studies were also followed through to spread litter once spread through the grow-out end of the shed. The presence of re-used litter in the grow-out end and new litter in the brooder end presented a "dual environment" with the shed presenting a "dual set of conditions". Importantly prior to chicken movement the piled and then spread re-used litter did not support any pathogen increase. Once the chickens moved across, there was not much difference between *E. coli* and *Clostridium perfringens* levels within these environments. *Salmonella* did show a tendency for a lower impact on re-used litter in terms of the variety of serovars and levels present. *Campylobacter* only appeared late in the cycles on both litter types. Overall there was no marked difference between both these litter environments once chickens were placed as well as through the chicken cycle, indicating no negative effects from the re-used litter.

It should be emphasised that effective control of food safety pathogens can be achieved and require the support of good in-shed management of both the environment during the chicken production cycle and the litter piling process between cycles. The data generated from these studies have been used to provide the basis of a code of practice which should be fully developed with subsequent industry consultation.

Introduction

In Australia, almost all meat chickens are raised in sheds on litter. The floor of the shed is covered by a layer of bedding material which absorbs moisture. During the growth cycle, this bedding material is mixed with excreta, feathers and wasted feed by the stirring action of the chickens and becomes litter.

Around 70% of the chickens placed in Australia are grown on single batch litter. In this system, the sheds are cleaned out after each batch of chickens the litter disposed and replaced with new bedding material. The remaining 30% of the chickens are grown on multi batch litter, usually under the partial clean out method. In the partial clean out approach, the litter at the end of the batch is moved from the brooding end of the shed into grow-out end. Alternatively, in some systems, the litter at the brooder end is removed from the shed and sent off-farm. The litter remaining in the shed is then be heaped and subjected to a short composting period (4 – 10 days) and then spread in the grow-out end prior to chick placement. Clean bedding material is spread in the brooder end of the shed.

Management of poultry litter from sourcing the initial bedding material through to utilisation of spent litter (litter end-use) is a concern to the Australian poultry industry and community (Runge et al. 2007). Traditionally, poultry litter has been used as a fertiliser in intensive horticulture, citrus and cropping industries as well as in broad acre agriculture.

There is a range of materials that have been used as bedding material by the Australian poultry industry. Typically, materials that are by-products from other industries have been used and include: timber shavings, hard- and soft-wood sawdust, rice hulls, shredded paper, sunflower husks, woodchips, chopped straw and tea-tree fibre (the material left after the extraction of tea-tree oil). The type of material used as bedding depends on what is available, suitability and cost in the locations where the chickens are grown. Cost variation largely reflects availability and transport distances.

The volume of bedding material placed per unit area of house floor space varies according to the type of bedding material, depth of bedding desired and how much the material settles when spread on the floor (Runge et al. 2007). Moisture content of the litter is the main effect on the volume of material removed from a shed as damp litter tends to compact more than dry (friable through to dusty) litter. In approximately seven weeks, 1,000 meat birds produce approximately 1.5 tonnes of dry poultry litter comprising of 50% litter and 50% manure.

Runge *et al*. (2007) estimated that there was approximately 4,274,000 m^2 (more than 2,750 sheds) of shedding for meat production in Australia. By assuming there is 5.5 batches per shed per annum about 1.17 million m^3 of bedding material is used producing approximately 1.60 million m^3 of spent litter for utilisation. These estimates do not take into consideration the effects of litter decomposition on the volume of material produced. The volume of spent litter removed at the end of a batch is generally 1.5 – 2 times the volume of material originally placed in the shed (Runge et al. 2007).

There have been concerns expressed that chickens raised on multi-batch litter may be exposed to higher levels of food-borne pathogens (such as *Campylobacter* and *Salmonella*) than chickens raised on freshly placed bedding material (i.e. single use litter). This concern is not based on actual scientific data – simply a perception that multi-batch litter must contain such pathogens and that these pathogens can colonise the chickens and increase the load of food-safety pathogens in food chain. This view then leads to the suggestion that the risk of food-borne pathogens in the food chain can be reduced by simply ensuring that all meat chickens are placed in sheds that contain fresh bedding material (i.e. raised in single use litter systems).

The possible removal of the option to adopt multi-batch litter systems is of concern for the poultry industry. A situation where 100% of meat chickens were raised on fresh bedding material would greatly increase the demand for this material and would also increase the amount of litter to be removed from chicken meat sheds. At the same time, there are increasing levels of restrictions on the approved end-uses of chicken litter.

Economic Value

The Australian Chicken Meat Industry produces around 500 million birds (700,000 tonne of chicken meat) annually and has an annual retail value in excess of $3.6 billion. In 2003, chicken meat consumption per capita in Australia reached 34.1 kg. Consumption is forecast to rise to 36.5kg in 2005/06 – meaning that chicken would replace beef as the most popular meat of Australian consumers. Consumption of chicken meat has increased 27% over the past decade, and is expected to continue increasing at between 1-5% pa for at least the next five years. Approximately 98% of the total chicken meat production in Australia is consumed domestically.

The chicken meat industry has indicated that there is a high priority on the need to develop valid guidelines to ensure that the practice of multi-batch litter use can remain a management option for the sustainable production of a major food.

Pathogens of Relevance

Based on the available information in the literature, the pathogens of key importance in the issue of litter re-use are *Salmonella* and *Campylobacter*. Both of these pathogens have the potential to enter the food chain via the production system. The accepted means of transmission from chicken-to-human for these two pathogens is predominantly via the food chain or less commonly by direct chicken faecal-human oral transmission.

Pathogen Reduction

Pathogen reduction can occur in litter piles held for 1-2 weeks as a partial composting will occur. It is important to understand that litter piling is not composting. Composting is defined as a process in which the litter is mixed with a carbon source and then either passive or actively aerated over a period of 10-12 weeks.

Pathogen die-off

With either litter piling or full composting, there is a range of potential mechanisms that could be detrimental to pathogen survival in litter. The impact of these mechanisms could vary depending on whether the pathogens are subjected to a full, partial composting or simply a pile-up process. Such mechanisms include heat, microbial competition or nutrient destruction/depletion, and antagonism from indigenous microorganisms and time. Of these potential mechanisms, it is generally accepted that temperature and time are the major effects in pathogen killing. Antagonism from indigenous organisms and nutrient destruction/depletion are regarded as the major mechanisms of ensuring that pathogens do not re-grow.

A widely accepted rule is that a minimum temperature of 55^{o}C for at least three days would result in highly efficient kill of pathogens. An alternative guideline of 60^{o}C for 30 minutes has been suggested as a better basis for ensuring pathogen killing.

Pathogen Re-growth

Pathogen re-growth is a newly emerging area of concern. Attention in this area has focussed on the fact that several studies have demonstrated that surviving *Salmonella* organisms in stored compost can re-grow to levels that pose health hazards. Recent work has suggested that the main control force preventing or limiting the re-growth of pathogens (*Salmonella* specifically) in compost is the indigenous microbial population (Sidhu et al. 2001).

As the indigenous population of compost declines with prolonged storage, the potential for *Salmonella* re-growth increases with prolonged storage of compost. Hence, the current recommendation of (Sidhu et al. 2001) is to have only minimal storage of compost after maturation has been achieved.

Litter Piling

The intention of litter piling (also called partial composting and deep stacking) is to achieve pathogen reduction/killing. The time period typically used in this technique means that there is no major effect on C:N ratio or the other nutrient aspects of full composting.

Conclusions

Overall, there is considerable literature evidence that the litter pile up process that is used by Australian producers has the potential to contribute to pathogen die-off, even though the piling process is of a short duration of around 7 days. However, there is no publicly available evidence of the effectiveness of the litter piling process in the Australian context.

The present set of trials have been designed to

- Validate microbiological methodologies used to assess pathogen survival in piled litter
- Assess the die-off of key food-borne pathogens during the pile-up process
- Assess the potential of the shed to support pathogens during a chicken cycle under re-use operations

Objectives

The objectives of the research were as follows:-

1. Perform shed trials to evaluate current practices for pathogen reduction in used chicken litter
2. Perform laboratory/pen trials to evaluate novel/alternative practices for pathogen reduction in used chicken litter
3. To establish simple physical parameters to monitor pathogen reduction
4. Identify effective and suitable pathogen reduction strategies

While undertaking the research it became obvious that current pile –up practices were effective and suitable for pathogen reduction. Hence the project progressed to carry out studies to assess the potential of the re-used litter (once spread) to support pathogens during a typical chicken production cycle.

Methodology

This Chapter has been set out to provide an overview of the general methodological approach used in this project. In the following chapters, detailed methodologies that are specific for the various field and laboratory studies (microbiological, physical and field) were evaluated.

Industry Consultation

Regular consultation meetings were held with Dr Margaret McKenzie and Mr Gary Sansom. The initial industry consultations emphasised that the main concerns were the possible transfer of food-borne pathogens across the broiler cycles. This confirmed that the focus of the work is to develop an understanding and knowledge that will assist the industry in minimising the food-borne pathogen risk across broiler cycles when re-using litter.

Discussions were also held in relation to the following:

- Litter material used
- Turn-around times
- Distribution and location of farms willing to participate in the trials
- Clean-up procedures adopted following pick-up

The bedding material used by farms is variable. Since the most common material used in Queensland broiler sheds is pine shavings, it was decided to use farms that used pine shavings for the trials.

The typical turn-around time between broiler flocks is 7- 10 days. However, the time is dependent on a number of variables – which include overall company policy; the market demands and the supply of available birds.

With industry input, it was agreed to initially assess the impact (on food-borne pathogen levels) of the current litter re-cycling practices commonly adopted by industry. Based on the outcomes of these initial studies, the latter stages of the project will explore alternative approaches (using both field and laboratory experiments) to minimise the food-borne pathogen risk across broiler cycles.

Farm Identification

Consultation with industry resulted in the identification of two farms suitable for this study. One farm is located at Donnybrook (to the north of Brisbane) and the other farm is located at Laravale (south-west of Brisbane). The owners and managers of both farms willingly supported and assisted the work. These two farms had a long history of litter re-use and welcomed the approach of providing a scientific basis to their current practices.

Overview of activities

The work of this project consisted of two phases.

The first phase dealt with developing a preliminary understanding of the physical and microbiological issues surrounding the testing of litter both in field based and laboratory based studies. This work was essential to the development of sound experimental design for the second, on-farm phase.

Areas of activity in the first phase were as follows:-

A) Evaluating suitable methods for assessing physical parameters of litter.
   - Litter moisture
   - Litter pH
   - Litter temperature
   - Litter water activity

B) Validating microbiological methods
   - Designing suitable samplers for collecting samples from the core of large litter piles as well as the surface of the piles
   - Validating microbiological methods

C) Using simple laboratory based methods to understand the survival pattern of pathogens under laboratory conditions
   - Survival pattern of *Salmonella* (and *E. coli*) in relation to pH and temperature

D) Preliminary field trials to aid in developing experimental design to test pathogen variation across broiler cycles
   - Simple field sampling to understand pathogen levels as soon as chickens have been removed
   - Simple field trial to understand the dimensions of a litter pile and assess variations across a pile and depths to which sampling will need to be undertaken as well as develop a random design for sample collection from a pile
   - Simple field trial to validate moisture assessment techniques

The second phase of the project consisted of on-farm trials. These studies involved trials of the ability of existing techniques (litter piling) to reduce the levels of *Campylobacter* and *Salmonella* in the litter. Thus no modification was done to the existing practices adopted by the individual farmers.

During these trials, physical parameters (pH, moisture, temperature) were evaluated as potential tools for monitoring/predicting pathogen reduction. Hence, the piles were intensively monitored for both physical and biological parameters. These parameters were moisture, temperature, water activity as well as levels of *E. coli*, *C. jejuni/coli*, *Salmonella* spp., *Clostridium perfringens* and *Bacillus* spp.

This was followed by studies on spread (re used) litter during a chicken production cycle.

The detailed methods and results of the first phase activities are presented in Chapter 1. The detailed methods and results of the second phase activities are presented in Chapters 2. The basis for the codes of practice (to be finalised with industry) is dealt with in Chapter 3.

Chapter 1

Laboratory and Field Validation Studies

Introduction

The work described in this Chapter was aimed at validating both microbiological methods (for pathogen enumeration) as well as physical methods (for establishing profiles of parameters such as temperature and moisture content).

Materials and Methods

Litter

When performing laboratory based experiments, litter was obtained from near the end of a broiler cycle on either the Laravale or the Donnybrook farm.

Bacteria

All bacteria used in spiking experiments in this Chapter were field isolates originally obtained from broiler litter. The bacteria used were *Salmonella enterica* serovar Sofia and *Salmonella enterica* serovar Virchow. The isolates had been serotyped at the Institute for Medical and Veterinary Science in Adelaide. The isolates were kept stored at -7ºC and were revived as fresh sub-cultures as needed. The *Salmonella* isolates were grown on sheep blood agar or in tryptic soy broth.

Laboratory Survival Experiments

Freshly collected litter was weighed into replicate sterile plastic containers. In some experiments, the containers were kept open (to simulate aerobic conditions) or closed (to simulate anaerobic conditions). In some experiments, additional water was added to the containers to simulate high moisture conditions. As well, different incubation temperatures were used.

Enumeration methods

Brief details of the various enumeration methods used in this study are provided in the following subsections.

E. coli

E. coli enumerations were performed by a direct count method on Petrifilm (3M) and/or Chromocult Agar (Merck) (using both the original formulation and the new selective formulation). All incubations were in air at 36.5ºC.

Salmonella

Salmonella enumerations were performed using a three tube MPN method. The non-selective enrichment broth – buffered peptone water (BPW) – was incubated at 37ºC. After this overnight incubation in BPW, each BPW was inoculated onto a semi-solid agar (MRSV, Oxoid) and the plate incubated at 42°C. Any MRSV plates showing a white haze surrounding

the inoculated drop (indicating the presence of motile salmonella) were sub-cultured (from the outer hazy zone) onto XLD agar (Oxoid). In some work, XLT4 (Oxoid) plates were used in addition to the XLD plates. The XLD and the XLT4 plates were incubated at 37ºC. Presumptive *Salmonella* isolates were confirmed using the Obis kit (Oxoid) and by agglutination using a commercial polyvalent O antiserum (Difco). Most confirmed *Salmonella* isolates were then submitted to the Salmonella Reference Laboratory at the Institute for Medical and Veterinary Science for full serological identification.

A number of litter samples were examined using an Immunomagnetic Separation (IMS) method in parallel with a standard three tube MPN method as described above. In this approach, each incubated BPW was also subjected to IMS as described by the manufacturer (Dynal). The IMS material was then used to inoculate MSRV plates as described above.

Campylobacter

The presence and number of *C. jejuni/coli* was determined using a three-tube MPN method. The selective enrichment broth used was Preston Broth (Nutrient broth No: 2 with antibiotics - Oxoid). The incubation of enrichment broths was at 37ºC for 4 hours and then 42ºC for 44 hours. Following enrichment, the tubes were inoculated directly onto modified Campylobacter charcoal deoxycholate agar-Preston – CCDA (Oxoid). In some experiments, the sub-culture was done after 24 and 48 hours of enrichment while in others, only the 48 hour sub-culture was used. The agar plates were incubated at 37ºC for 48 hours. All incubations were done under an atmosphere of 90% N_2, 5% CO_2 and 5% O_2 provided by a commercial sachet system (Oxoid). Presumptive *Campylobacter* isolates were then subjected to confirmatory phenotypic tests (catalase, oxidase, motility, characteristic cell shape) as previously described (Chinivasagam et al. 2004).

Staphylococcus spp.

The presence and number of *Staphylococcus* spp. was determined using a direct spread plate method on Baird Parker Agar (Oxoid). The plates were incubated aerobically at 37ºC for 48 hours.

Bacillus spp.

The presence and number of *Bacillus* spp. was determined using a direct spread plate method on *Bacillus cereus* Agar (Oxoid). The plates were incubated aerobically at 30ºC for 24 hours.

Initial Litter Pile Study

This study was performed on the Laravale farm. There were two litter piles in the study shed: a single use litter pile (from the brooder end of the shed) and a multiple-use litter pile (from the grow-out end of the shed). Figure 1.1 shows the physical dimensions of both piles. Both piles had been in place for two days.

Both surface and core samples were collected at various positions from each pile (see Figure 1.2). For both piles, surface litter was sampled to a depth of 10 cm from the surface. For both piles, the surface samples were collected along the length of the pile. Seven horizontal surface samples were collected from the multi-use pile and three from the single use pile. As well, a vertically distributed set of surface samples (ie down the slope face of the pile) were collected from each pile. These vertically distributed samples were collected at 60 cm, 120 cm, 180 cm and 240 cm from the top of the pile.

Core litter samples were collected at two points in both piles using a grain sampler. The core samples were collected at 120 cm from the top of the pile.

Microbiology

For *Campylobacter, Salmonella* and *Bacillus* all surface samples of the single use pile were pooled and examined as a single sample. The two core samples were similarly pooled and examined. The same approach was used for the multi-use litter pile.

For *Staphylococcus*, the horizontal surface samples from the single use pile were pooled and examined as a single sample. The vertical surface samples of the single use pile were similarly pooled and examined as a single sample. The core samples of the single use pile were pooled and examined as a single sample. The same approach was used for the multi-use litter pile.

For *E. coli* all surface samples were examined separately. Within each pile type, the core samples were pooled.

Physical measurements

For moisture content, all vertical surface samples (from both piles) were examined separately. For the single use pile, the three horizontal surface samples were pooled. For the multi-use pile, the horizontal surface samples were combined into two pools. For both piles, the core samples were pooled. For, water activity and pH measurements, all surface samples were examined individually. The core samples (within a pile type) were pooled.

Moisture content was determined in a standard laboratory assay. The moisture samples collected were stored without air chilled until tested for moisture content. The moisture content was defined as the difference in weight of the litter sample before and after drying at 100ºC for 18 hours.

The water activity was determined in the field using a water-activity meter. - *Safe Storage Quick Check.*

The pH of the litter samples was determined by suspending 2 g of litter in 50 ml of distilled water. After allowing the suspension to stand at room temperature for 5 minutes, the pH was measured with a standard laboratory pH meter.

Litter temperatures were measured using a thermometer at different locations.

Comparison of moisture measurement techniques

Two different moisture measurement techniques were used in this work. The two methods were used to evaluate the moisture content of litter in shed containing broilers at 49 days of age.

Five different bays within the shed were selected – Bays 5, 10, 15, 20 and 25.

A field instrument designed to provide real-time moisture measurements of soil – a Theta Moisture Probe – was used in the field. The Theta probe was inserted to a depth of 4 cm and the moisture content recorded. The probe was used to take five different readings – close to the drinker line at each of the bays.

As well, five litter samples – to a depth of 4 cm and a minimum volume of 200 gm – were collected from each bay. These litter samples were taken to the laboratory and subjected to standard moisture content analysis as detailed above.

Results

Evaluation of microbiological methods

The results of comparing the various microbiological methods using litter samples collected at Days 35, 49 and 56 of the broiler cycle are presented in Table 1.1.

There were no marked or consistent differences between the results obtained with any of the three methods used for estimating *E. coli* levels.

The two different *Salmonella* selective agar plates, XLD and XL4T, gave identical results.

The week old litter, deliberately left to age to increase stress on any *Campylobacter* present, gave the same count (0.3 MPN/g) in both MPN methods (with the difference in the MPN methods being the time of sub-culture from the enrichment broth – either after 24 hours or 48 hours).

A total of five different litter samples (both single and multi-use litter) were examined by both IMS and conventional MPN methods for *Salmonella*. The results are presented in Table 1.2. The IMS results were generally very similar to the results obtained by the conventional MPN method. Both litter samples that were negative by the conventional MPN were also negative in the IMS method. One litter that was a low positive in the conventional MPN method (1.1 MPN/g) was negative (<0.3 MPN/g) in the IMS method. Overall, there was no marked difference between the conventional MPN and the IMS supplemented MPN method.

Survival of *E. coli* in poultry litter under laboratory conditions

In this work, fresh litter and fresh litter with added water (to simulate wet litter conditions) were used. The litter samples were held in open or closed containers and incubated at 37ºC for 48 hours, then 45ºC for 48 hours and then 55ºC for 48 hours (to simulate a rising temperature within a litter pile). The initial *E. coli* count was 10^5 CFU/g. No *E. coli* was detected in any of the subsequent sampling times (samples were taken at every 48 hours) despite the minimum detection level being 10^3 CFU/g.

Survival of *Salmonella* in poultry litter under laboratory conditions

In this work, samples of fresh litter were held in plastic containers at 37ºC, either with the lid on (simulating anaerobic conditions) or lid off (simulating aerobic conditions). The treated containers were spiked with an overnight culture of two different strains of *Salmonella*. The initial spike resulted in a *Salmonella* count of 1.67 X 10^8 CFU/g. After three days of incubation, the *Salmonella* count in the "open" container was 110 MPN/g – a reduction of around 10^6. No *Salmonella* was detectable in the "closed" container. As the minimum detection of the MPN method was 0.3 MPN/g, the reduction in *Salmonella* in the "closed" container was at least 10^8.

The pH of the initial litter was 9.43. After three days of incubation, the "open" container had a pH of 8.23 while the "closed" container had a pH of 9.3.

In a second experiment, spiked litter samples were held in "open" and "closed" containers that were incubated at 45ºC. In this experiment, the spike was only a single strain – *S.* Sofia. The six spiked containers had an initial *Salmonella* count of 1.67 X 10^5 CFU/g. Three of the spiked containers were incubated with the lid off and three were incubated with the lid on.

After 24 hours incubation, no *Salmonella* could be detected (minimum detection limit of 0.3 MPN/g) in any of the six spiked containers.

The initial pH of the litter was 8.42. Following incubation, the "anaerobic" (lid on) litter had a similar pH while the "aerobic" (lid off) litter had increased to 8.91.

Typical biological and physical parameters of litter just after final pick up

The levels of key bacteria and the temperature of litter were determined immediately after the final chicken pick-up on the Donnybrook farm. For this third consecutive broiler cycle, the brooder end of the shed had single use litter while the grow-out end had the multi-use litter.

The levels of the various bacteria are shown in Table 1.3. As well, the litter was held for one week at ambient in the laboratory and then re-examined for *Salmonella* and *Campylobacter* levels.

There was no marked difference in the *E. coli* levels in the single use and re-use litter (see Table 1.3).

Campylobacter was absent in both single use and re-use litter but *Salmonella* was present at a low level (0.7 MPN/g of litter) in the single use litter and absent from the re-use litter (see Table 1.3).

The stored litter showed no marked difference in *Salmonella* levels (see Table 1.3).

The *in situ* temperatures recorded for the litter are shown in Table 1.4. The results are an average of a multiple readings. Whilst not conclusive, it is interesting to note that the litter temperatures in the re-used litter were slightly higher than those present in the single use litter.

Initial litter pile study

Considerable difficulty was experienced in obtaining core samples within the litter pile using the grain sampler.

The results of the sampling for *Campylobacter, Salmonella* and *Bacillus* are set out in Table 1.5. While no *Campylobacter* were detected in any sample, a low level of *Salmonella* was detected in the surface sample from the multi-use pile.

There was no detectable *E. coli* (minimum detection level was 100 CFU/g) in any of the surface or core samples from the single use litter pile. Similarly, there was no detectable *E. coli* (minimum detection level was 100 CFU/g) in any of the core samples from the multi-use litter pile. However, there were detectable levels of *E. coli* in some of the surface samples in the multi-use litter pile (see Table 1.6).

The *Staphylococcus* levels did not show much variation – either between single or multi-use piles or from surface to core samples (Table 1.7).

The results of the physical measurements (moisture content, water activity and pH) are shown in Table 1.8.

Comparison of moisture measurement techniques

The results of the comparison of moisture measurement techniques are presented in Table 1.9.

Discussion

The purpose of the preliminary work described in this Chapter was to validate the various physical and microbiological methods and to provide some basic information to guide the final design of the formal, full-scale field trials.

From the various methodologies tested, suitable methods were identified for quantifying the key organisms in litter. Direct plating using Chromocult was adopted in favour of using Petrifilm for *E. coli*. There was little difference between the use of immunomagnetic separation following primary enrichment (BPW) and the use of selective enrichment (MSRV) for isolating *Salmonella*. Hence, the conventional selective enrichment was adopted for *Salmonella*. *Campylobacter* was successfully isolated using enrichment in Preston broth followed by plating onto mCCDA agar. For both *Salmonella* and *Campylobacter,* a MPN approach was adopted to quantify these two key organisms in litter.

The laboratory work showed a very poor survival of *E. coli* in poultry litter under laboratory conditions – with levels dropping at least by 100 within 48 hours of the litter being held at the relatively low temperature of 37ºC (regardless of any addition of moisture or whether the litter was held in open or closed containers). This poor survival could have been due to the development of volatile compounds such as ammonia, other microbial interactions within the litter or combinations of these factors. These studies will need to be validated in the field for litter piles in sheds.

A somewhat better survival occurred with *Salmonella.* Detectable levels of *Salmonella* were present in litter incubated at 37ºC for three days. The reduction in *Salmonella* levels was still marked, at least by 10^6. The drop in *Salmonella* counts was also evident when an incubation temperature of 45ºC was used – with a drop of at least 10^6 in both open and closed litter containers.

Clearly, litter pH (as well as other factors such as moisture content and water activity) impact on the survival of *Salmonella* (and *E. coli)* in litter. The laboratory based studies indicate that levels of both of these organisms are likely to fall markedly in piled litter in sheds. However, study under field conditions using actual litter piles in sheds are required to confirm these laboratory studies.

As part of the general preparation for the field studies, the levels of key bacteria in litter were determined immediately after the final chicken pick-up on one of our two study farms. The levels we found of *E. coli* and *Salmonella* were in the expected range based on the prior national litter survey (Chinivasagam et al. 2008a).

As noted in the Introduction section, the issue of the re-growth of *Salmonella* is now an issue of concern in bio-solids. Litter samples were stored for a week in the laboratory at ambient temperature to understand the possibility of re-growth of *Salmonella* under these conditions. The results (see Table 1.2) indicate that there was no marked re-growth of *Salmonella* in litter stored in this manner.

Staphylococci are commonly found in litter (Lu et al. 2003). The presence of these organisms in litter is presumably due to the association of these organisms with chickens. The finding that these organisms are present in different litter pile locations means that these organisms are well adapted to persist in piled litter.

The initial pile study demonstrated that a grain sampler was not a good instrument for sampling deep within a litter pile. The litter is not as heavy as grain and thus does not easily collect in to the grain sampler (as in a silo). As a result of the problems with the grain sampler, a special sampling device that can be driven into the centre of the pile using a heavy mallet has been developed and will be used in the full field trials.

The initial litter pile study found a degree of variability in the *E. coli* levels present at various positions (both horizontal and vertical) in the surface of the pile. This finding is important in guiding the sampling strategy to be used in the full field studies. Full field studies will be designed in a manner to ensure that enough samples are collected to compensate for this variability across the pile when compositing samples. It is notable that the major variation detected occurs down the slope surface (with counts of *E. coli* varying by at least 100 fold).

The physical measurements taken in the initial litter pile study provided valuable insight. The areas of high water activity seem to be contained to the core of the litter pile rather than the surface. However *Salmonella* spp were isolated from the surface samples. This preliminary study indicates that a good understanding needs to be developed between water activity, pH and temperature in relation to bacterial survival (and /or growth). An understanding also needs to be developed of the microbial interactions between the pathogens and other inherent flora. All these need to be understood in terms of volatiles produced such as ammonia – which probably are a contributing agent to the high pH seen in litter.

Thus such information will enable the development of suitable litter pile management / manipulation techniques in managing pathogen reduction between litter cycles.

It should be noted that the water activity was tested by collecting samples in the field, transporting them to the laboratory and then doing the water activity determinations. It was felt that this delay in analysis may mean that the measurements do not accurately reflect the situation in the field. During the field trials the water activity will be measured on-site directly from the piles.

Similarly litter temperatures will be logged electronically which will be a more convenient and accurate way to monitor temperature. The data generated ensured the purchase of suitable electronic data loggers.

There was a lack of correspondence between the simple field measurements of litter moisture obtained by use of Theta probe and the accepted "gold standard" laboratory assay. This failure of the Theta Probe probably reflects that the instrument was developed for soil moisture applications. The much less dense nature of litter when spread on the shed floor is a problem for this instrument. Thus, though not convenient, laboratory based methods for moisture measurement will be used in the field trials.

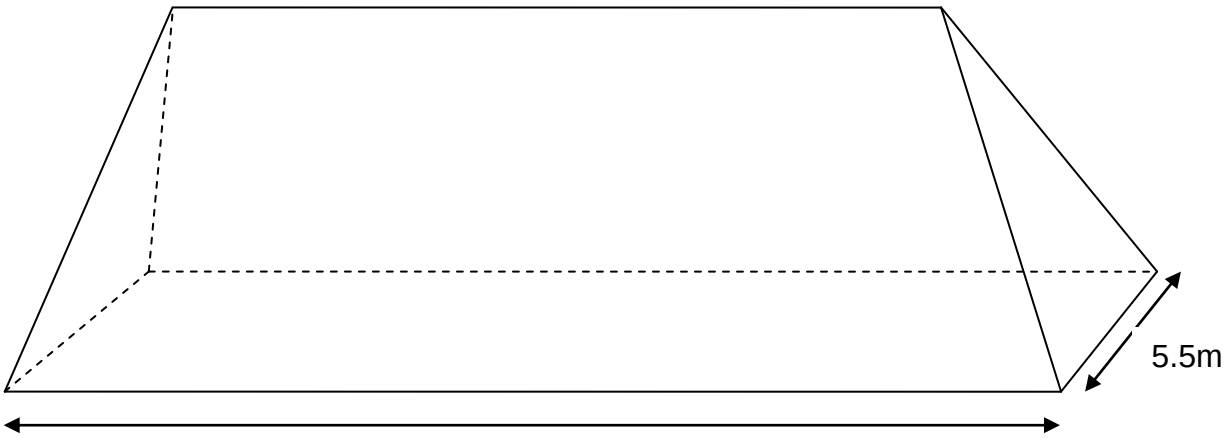
Overall the following was achieved in these preliminary studies:-

- Microbiological methods for pathogen enumeration were validated
- Moisture assessment methods were validated
- A technique for determining water activity was established
- Pathogen die-off potential was assessed both in the laboratory as well as during random shed sampling
- The distribution of *E. coli* in different positions in the litter pile was understood to develop protocol for composite sample preparation

- A suitable sample collection strategy for collection of core sample by the development of core sampler specifically for collecting litter from piles was developed

Figure 1.1 Dimensions of litter piles (Laravale farm; August 2006)

Single Batch Pile



Multiple Batch Pile

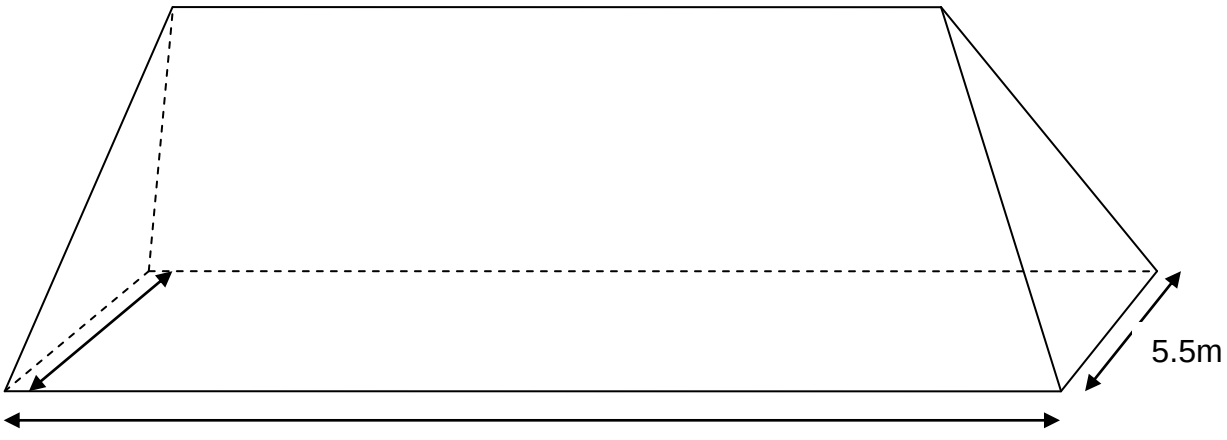
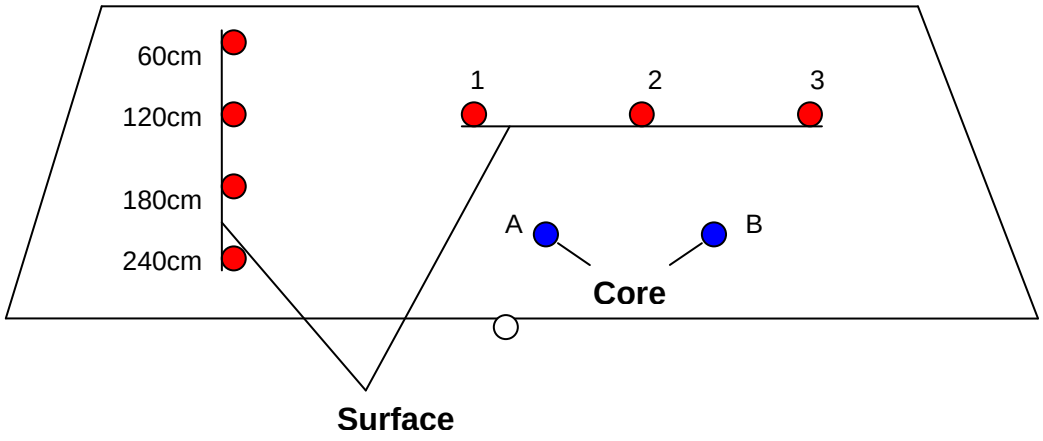


Figure 1.2 Layout of sample sites on litter piles (Laravale farm, August 2006)

Single Batch Pile



Multiple Batch Pile

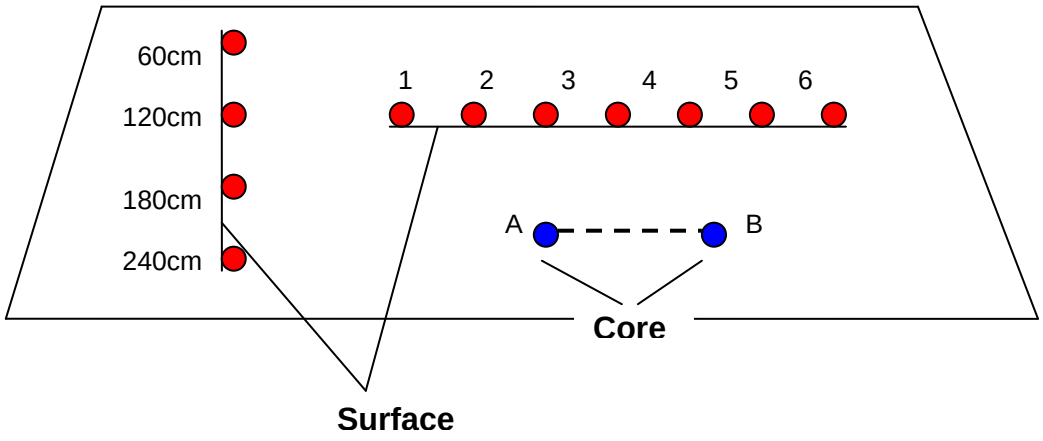


Table 1.1 Levels of bacteria in litter collected from the study shed at the indicated day of the broiler cycle

Cycle day	*E. coli* cfu/g			Coliforms cfu/g			*Campylobacter jejuni/coli* MPN/g		*Salmonella* MPN/g
	Petrifilm	Chromocult	Chromocult (Selective)	Petrifilm	Chromocult	Chromocult (Selective)	24 hours - enrichment	48 hours - enrichment	
Day 36	330,000	570,000	540,000	570,000	130,000	310,000	0.3[A]	0.3[A]	0.3[B]
Day 49	580,000	87,000	NT[C]	380,000	130,000	NT	NT	>11,000	0.4 [B]
Day 56	1,800,000	4,000,000	NT	2,200,000	1,900,000	NT	NT	<0.3	<0.3 [B]

[A] These *Campylobacter* results were obtained on litter held at 4ºC for one week prior to testing.

[B] These are the results obtained using XLD agar. Identical results were obtained using XL4T agar.

[C] NT = Not tested.

Table 1.2 Results of comparison of conventional MPN and immuno-magnetic separation (IMS) in combination with MPN for the enumeration of *Salmonella*

Sample	Salmonella Result (MPN/g)	
	Conventional MPN	IMS/MPN
1	<0.3	<0.3
2	<0.3	<0.3
3	1.1	<0.3
4	1.1	1.1
5	1.5	0.7

Table 1.3 Levels of bacteria in litter and stored litter (Donnybrook farm, third cycle, July 2006)

Organism	Single Use Litter		Re-use Litter	
	Drinker Line	Feeder Line	Drinker Line	Feeder Line
Salmonella (MPN/g)				
Initial	0.7	ND[A]	<0.3	ND
After 1 week storage[B]	<0.3; <0.3	1.1; 1.1	0.3; 1.1	0.7; 1.5
Campylobacter (MPN/g)				
Initial	<0.3	<0.3	<0.3	<0.3
After 1 week storage[B]	<0.3; <0.3	<0.3; <0.3	<0.3; <0.3	<0.3; <0.3
E. coli (CFU/g)	4.3×10^4	ND	1.0×10^4	ND

[A] ND = Not Done
[B] The litter sample was held at ambient temperature for 1 week and then re-tested. The results of duplicate samples are shown.

Table 1.4 Litter temperatures (Donnybrook farm, third cycle, July 2006)

Temperature (ºC)	Single Use Litter		Re-use Litter	
	Drinker Line	Feeder Line	Drinker Line	Feeder Line
Mean	33.7	32.4	34.4	33.9
Maximum	32.9	32.9	34.7	33.7
Minimum	31.8	32.0	34.2	32.2

Table 1.5 Results of *Campylobacter*, *Salmonella* and *Bacillus* enumeration of litter pile samples (Laravale farm, August 2006)

Litter Sample and Litter Type	*Salmonella* MPN/g	*Campylobacter* MPN/g	*Bacillus* CFU/g
Core Single Use	<0.3	<0.3	210,000
Surface Single Use	<0.3	<0.3	360,000
Core Multi Use	<0.3	<0.3	52,000
Surface Multi Use	90	<0.3	66,000

Table 1.6 Results of *E. coli* enumeration for surface samples from the multi-use litter pile (Laravale farm, August 2006)

Sample Type	Position	*E. coli* Count (CFU/g)
Horizontal	1	<100
	2	400
	3	<100
	4	<100
	5	<100
	6	<100
	7	1,600
Vertical	60 cm	<100
	120 cm	<100
	180 cm	400
	240 cm	18,000

Table 1.7 Results of enumeration of *Staphylococcus* spp. in litter pile samples (Laravale farm, August 2006)

Sample	*Staphylococcus* spp Count (CFU/g)
Vertical Pooled Surface Single Use Litter	454
Horizontal Pooled Surface Single Use Litter	331
Pooled Core Single Use Litter	218
Vertical Pooled Surface Multi Use Litter	672
Horizontal Pooled Surface Multi Use Litter	280
Pooled Core Multi Use Litter	432

Table 1.8 Results of physical measurements of litter piles (Laravale farm, August 2006)

Litter Type	Sample Type	Position	% Moisture	Average % Aw (ºC)	pH
Single	Surface (Vertical)	60 cm	28.4	0.63 (22.2)	9.10
	Surface (Vertical)	120 cm	25.4	0.69 (22.2)	8.77
	Surface (Vertical)	180 cm	27.4	0.64 (22.2)	8.97
	Surface (Vertical)	240 cm	27.8	0.64 (22.2)	8.86
	Surface (Horizontal)	1		0.53 (22.4)	8.68
	Surface (Horizontal)	2	26.7 (composite)	0.62 (22.3)	8.92
	Surface (Horizontal)	3		0.50 (22.0)	8.83
	Core		21.0	0.92 (21.9)	8.80
Multi	Surface (Horizontal)	1		0.67 (22.6)	8.82
	Surface (Horizontal)	2	23.6 (composite)	0.61 (22.2)	8.97
	Surface (Horizontal)	3		0.52 (21.9)	8.98
	Surface (Horizontal)	4		0.63 (21.6)	8.86
	Surface (Horizontal)	5		0.57 (21.4)	8.79
	Surface (Horizontal)	6	24.8 (composite)	0.66 (21.2)	8.96
	Surface (Horizontal)	7		0.69 (21.1)	8.95
	Surface (Vertical)	60 cm	21.3	0.58 (21.1)	8.74
	Surface (Vertical)	120 cm	23.9	0.54 (21.2)	8.99
	Surface (Vertical)	180 cm	20.1	0.54 (21.3)	8.89
	Surface (Vertical)	240 cm	22.2	0.56 (21.7)	8.94
	Core		31.6	0.92 (22.0)	8.92

Table 1.9 Comparison of moisture measurement techniques on litter collected at Day 49 of broiler cycle

Sample Site (Bay Number)	Moisture % as determined by						
	Theta Probe						Laboratory
	Reading 1	Reading 2	Reading 3	Reading 4	Reading 5	Mean	
5	34	46	36	35	38	37.7	42.1
10	16	25	15	17	21	18.8	29.3
15	11	12	13	15	12	12.5	26.9
20	36	43	36	37	28	36.2	27.7
25	12	8.3	13	9	12	10.9	28.1

Chapter 2

Pathogen survival in litter

Litter or shed environments such as dust can harbor either *Salmonella* or *Campylobacter* or both at various stages over the chicken production cycle (Chinivasagam et al. 2008b). These two key pathogens are generally present in chicken and have the capability to persist under a combination of conditions that may prevail in various niches in the shed both during litter push-up and subsequent production cycles. However, *Campylobacter* is known to have a poor survival potential (Sanders et al. 2007), although it has been suggested that the organism has the ability to go into a viable but non-culturable state (Rollins and Colwell 1986). Such features can contribute either to the persistence or the elimination of either of these organisms during the treatment of litter within the shed. However, not only is there a lack of understanding on the survival mechanisms that may prevail in such environments, but there is also a lack of sufficient data with respect to the populations during treatment of litter intended to be reused within the shed.

An understanding will assist not only in the monitoring of in-shed processes and hygiene but also act as a measure of comparison for assessing the efficacy of treatment processes adopted. For example an understanding of the levels of an indicator organism such as *E. coli* could be used as an indicator of treatment efficacy. The issues of levels of target organisms as opposed to simple presence absence test is a key point, Santos *et al*. (2005) demonstrated that the Most Probable Method (MPN) both provided more information and was significantly more sensitive than the presence absence test procedure for *Salmonella* (in turkey faeces).

Previous studies carried out on pathogens in Australian chicken litter (both from single use and re-use farms) have shown litter to contain varying levels of *Salmonella* (10^3 – 10^5 MPN/g) and *Campylobacter* (10^3 – 10^7 MPN/g) through a broiler production cycle (Chinivasagam et al. 2008b) and can still be present shortly after post chicken pick-up (Chinivasagam et al. 2008a). This gives an indication of the extent of the populations of these organisms that can potentially be present in litter.

Thus this section specifically deals with the following:

- The die-off of pathogens during the push-up process that is commonly adopted by some segments of the industry prior to re-use
- The impact of pathogen survival through a cycle with the commonly adopted protocol following push-up i.e. the use of new litter in the brooder (for raising young chicks) end and the use of re-use litter in the grow out end

Some key physical parameters such as temperature, pH, water activity (aW) and moisture all have an impact on the litter macro-environment and thus directly or indirectly linked to pathogen survival. Water activity has been used as a means of limiting microbial growth (as well as chemical deterioration) (Barbosa-Canovas et al. 2007). Both pH, and water activity have been linked with the reduction of *Salmonella* in poultry litter (Payne et al. 2007).

Temperature also has an impact on pathogen survival and composting processes have been known to reach 58 – 69ºC (due to microbial activity) (Tiquia et al. 1996). Such temperatures are known to contribute to the die-off of *Salmonella*, *Campylobacter* and *E. coli*. However the duration and even distribution of temperature through a pile are deemed important.

Thus a combination of all factors may be contributing to key pathogen die-off. It thus could be hypothesised that in various situations these parameters could act singly or in combinations

to contribute to pathogen die-off. Needless to say these simple physical parameters – once links have been established to pathogen die-off - could be easily monitored and managed to create unfavourable conditions for pathogen survival or proliferation.

The aim of the present study was to:

- Produce microbiological data to enable an understanding of the survival patterns of the two key pathogens, *Salmonella* and *Campylobacter* and the indicator organism *E. coli*.
- Relate these survival patterns to physical parameters such as temperature, moisture, pH and water activity as a means of developing field based relationships to be able to predict/understand pathogen survival patterns during the pile-up process.
- Understand treated litter when used across a broiler cycle in the typical situation where this litter is used in the brooder end

The overall approach adopted was
(a) Follow pattern of pathogen die-off in piled litter across sequential pile-up processes
(b) Follow pattern of pathogens in litter during several broiler cycles on re-used litter
(c) Compare (a) and (b) across two farms in Queensland

Materials and Methods

Farm selection

The Donnybrook and Laravale farms described in Chapter 1 were used in these studies. Both farms were from same company and therefore the overall management styles were similar, although the details of the actual litter reuse practices differed slightly. The two farms were included to allow a better comparison of the impact of these slightly differing litter re-use practices. The farm studies commenced in October 2006 on both farms, and sampling was carried out alternatively on both farms between their respective cycles.

Litter management practices

For both farms, the first cycle began with a full clean-out and all new litter, thus a uniform starting point. Both farms used pine shavings as the bedding material.

On the Donnybrook farm, the practice is as follows. At the end of chicken cycle 1, all litter is piled. Prior to the placement of chickens, the piled litter is spread in the grow-out end of the shed and fresh bedding placed in the brooder end. At the end of chicken cycle 2, the grow-out end litter is removed and the brooder end litter is piled. This litter is then spread at the grow-out end and fresh bedding placed at the brooder end. After the third chicken cycle a full clean out occurs. Hence, on this farm litter is only ever re-used once. The first and second litter pile-up cycles were followed, the only available before the full clean out. The duration of the pile-up cycle was decided by the farm management based on chicken placement dates.

At the Laravale farm, there is a slightly different management practice. The first testing on the litter pile generated was after the first chicken cycle. The litter pile generated after the second chicken cycle was also tested. As this litter consists of all the litter from cycles 1 and 2, the pile is quite large. After the third chicken cycle, a portion of the litter from the grow-out end is removed prior to pileup. This third cycle was also tested. Further chicken and litter cycles follow, although no further pile studies were performed. The duration of the pile-up cycle was decided by the farm management based on chicken placement dates.

These practices for both Laravale and Donnybrook are illustrated in the Figure 2.1.

Sampling from litter piles

The physical dimensions of the litter piles were recorded.

Samples were collected from the piles using a formal randomised sampling method. Initially, the pile was divided into five segments. Using random numbers a single segment was selected and allocated to temperature measurements. This segment was not used for the collection of samples for other purposes.

The whole pile was divided into 50 cm segments along the length of both sides of the pile. Each of these segments was designated with a number. On each sampling day, a total of five sampling sites were identified by random numbers. The same sampling site was examined on both sides of the pile (termed sides A and B). A site once sampled was not sampled again.

At each selected sampling point, samples of litter were collected. The samples consisted of surface samples (to a depth of 10 cm) collected from near the top of the face of the litter pile and from near the bottom of the face of the litter pile. Within a side, the five top samples were composited and separately the five bottom samples were composited. A specially designed stainless steel core sampler (designed using the experiences from the validation work performed in Chapter 1) was used to obtain a 2 m deep sample at each sampling point. These five core samples were composited. At times a sub-core sample (at a depth of either 1 or 1.5 m) was also collected with this same corer. Figure 2.3 illustrates the sampling strategy.

To gain an overall picture, samples of the spread litter – both before the pile up process and after the pile up process were also collected. The samples were collected using a specially designed stainless steel sampler that collected litter to a depth of 4 cm over an area of 400 cm^2. The samples were collected at random spots under feeder lines and under drinker lines (with the two sources being held separate). When the shed cycle was such that the brooder section had new litter while the grow-out end had re-used litter, litter samples were collected from both ends of the shed prior to the pile-up process. For samples collected after the pile-up, only the grow-out end of the shed (i.e. the re-used litter) was sampled. Where possible, the spread litter was sampled prior to the entry of chickens into the grow-out area. Once collected the samples were stored chilled and transported to the laboratory. Microbiological and pH analysis as described below was carried out within few hours of arrival to the laboratory.

Figure 2.1 Litter management practices on Donnybrook and Laravale farms

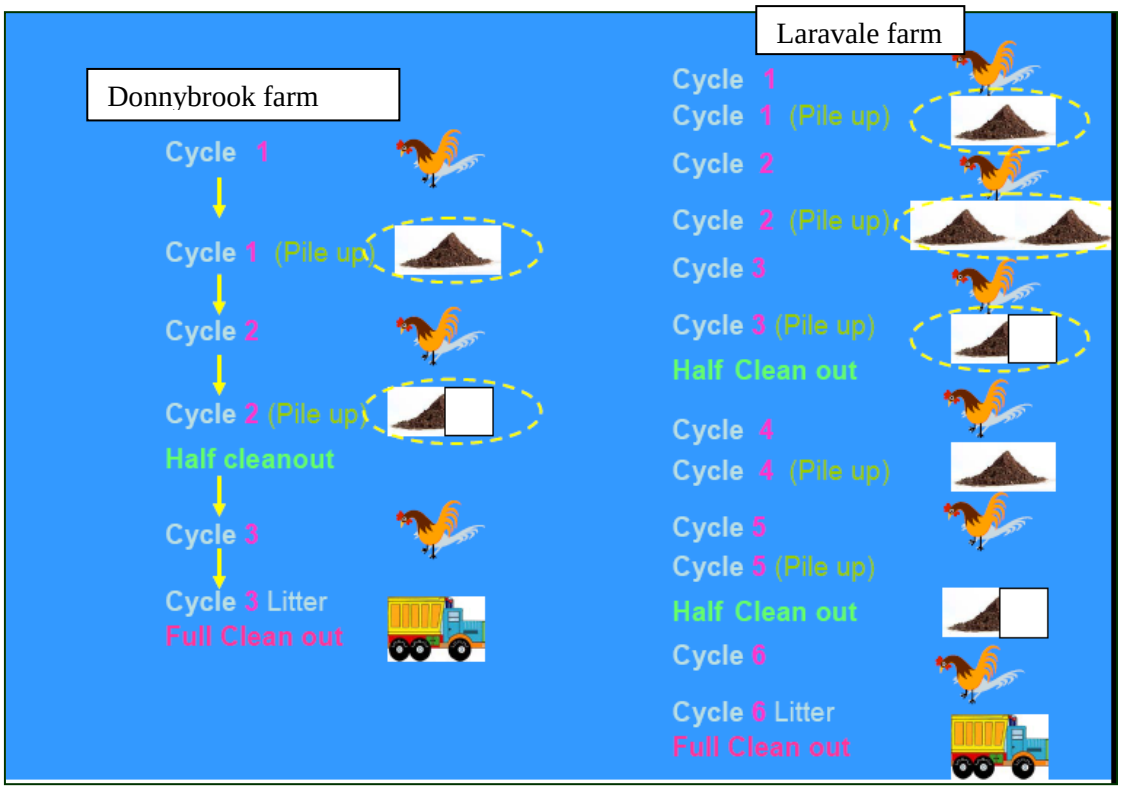
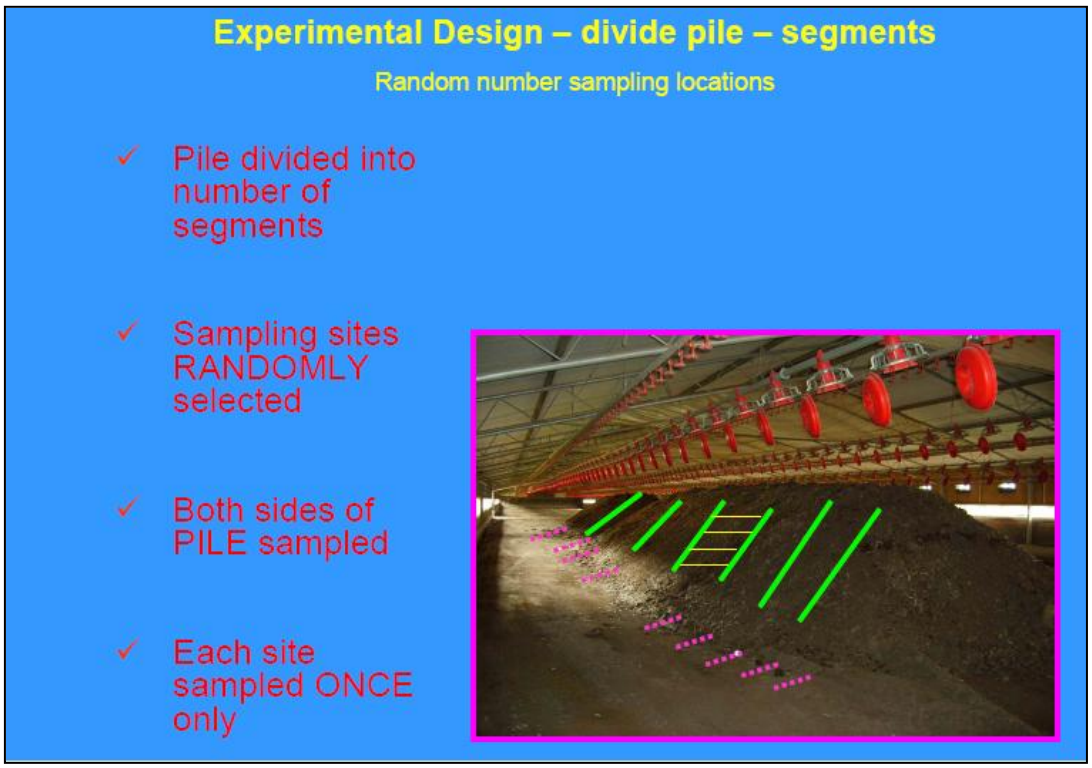


Figure 2.2 Sampling of litter pile. The green vertical lines indicate the five major segments of the pile. The horizontal yellow lines show the segment that has been randomly selected for temperature measurements. The purple lines show the 50 cm sub-segments marked along the entire length of the pile.



Temperature measurements

Temperature was measured continuously using a data logger (DataTaker DT80). For the litter piles, temperatures were measured near the surface (depth of 100 mm and 200 mm) near the top of the pile, in the mid-section of the pile and near the bottom of the pile at the three positions. Core temperatures at depths of 1 m, 1.5 m and 2 m, were measured at the mid-section of the pile and near the bottom of the pile. The actual positions used varied from pile to pile depending upon the availability of probes and pile dimensions. Figure 2.4 illustrates these locations.

Temperatures of spread litter were also measured using the DataTaker DT80. In this work, the temperature probes were placed into the spread litter in both the brooder (new litter) and grow-out (re-used litter) ends of the shed. As well, ambient air temperature was also recorded.

Sample collection from spread litter during a chicken cycle

This component of the trial is related to testing spread litter (both new and re-used) during a chicken cycle. Samples were collected from both the brooder and grow-out ends of the shed during a cycle.

Thus the shed was categorised into regions (see figure 2.5) as follows:

N1 – New litter - the brooder end that received new litter

N2 – New litter - the brooder end that received new litter

Buffer - this area was not tested due to the possible mixing of both litter and remained as a buffer zone where no samples were collected

R1 – Re-use litter - the grow-out end that received treated litter via the pile-up process

R2 – Re-use litter - the grow-out end that received treated litter via the pile-up process

The shed was divided according to the different bays along the length of the shed. At each sampling date, three bays were randomly selected within each sampling zone (N1, N2, R1, R2). Within each bay, three litter samples were collected. The samples were collected using a specially designed stainless steel sampler as described above. Once collected the samples were stored chilled and transported to the laboratory. Microbiological analysis and pH (as described below) was carried out within few hours of arrival to the laboratory. For water activity, each subset of three samples within a bay was tested. For microbiology, moisture content and pH, all nine samples within a sampling zone were composited. As well, the depth of litter was measured at each sampling site (three measurements per site).

Figure 2.3 Illustration of the sampling plan used for each daily sampling of litter piles.

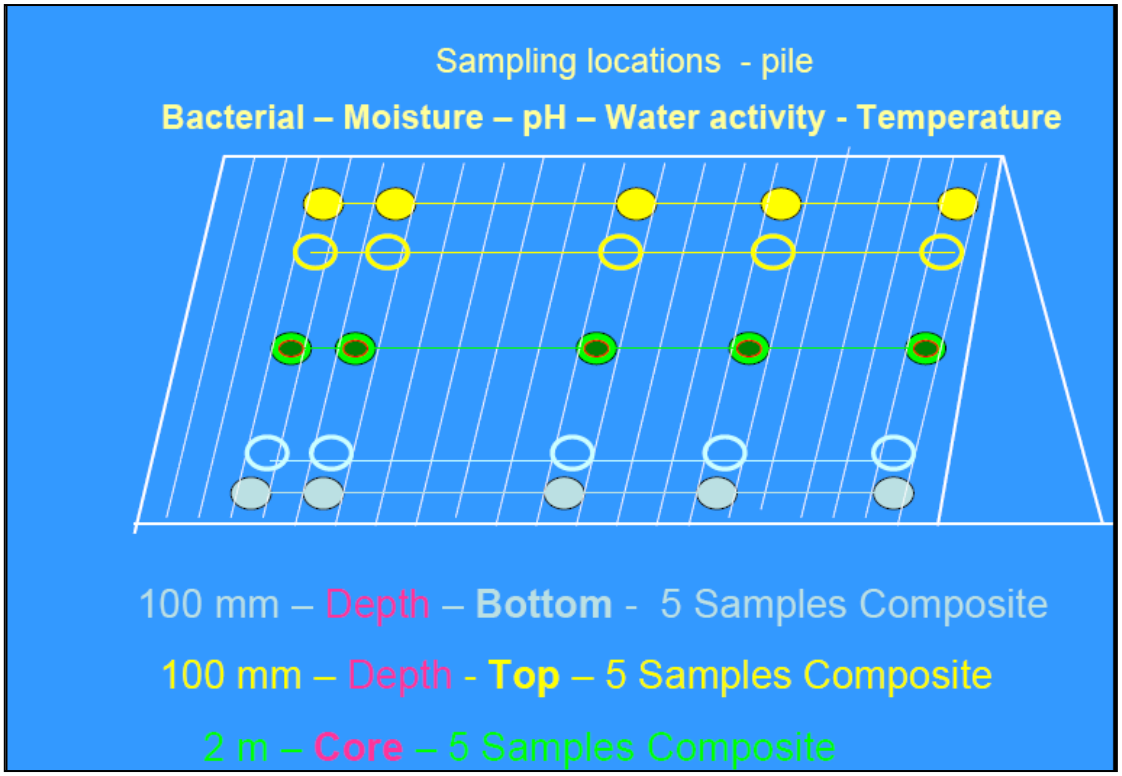


Figure 2.4 Illustration of the sampling plan used for temperature monitoring of litter piles.

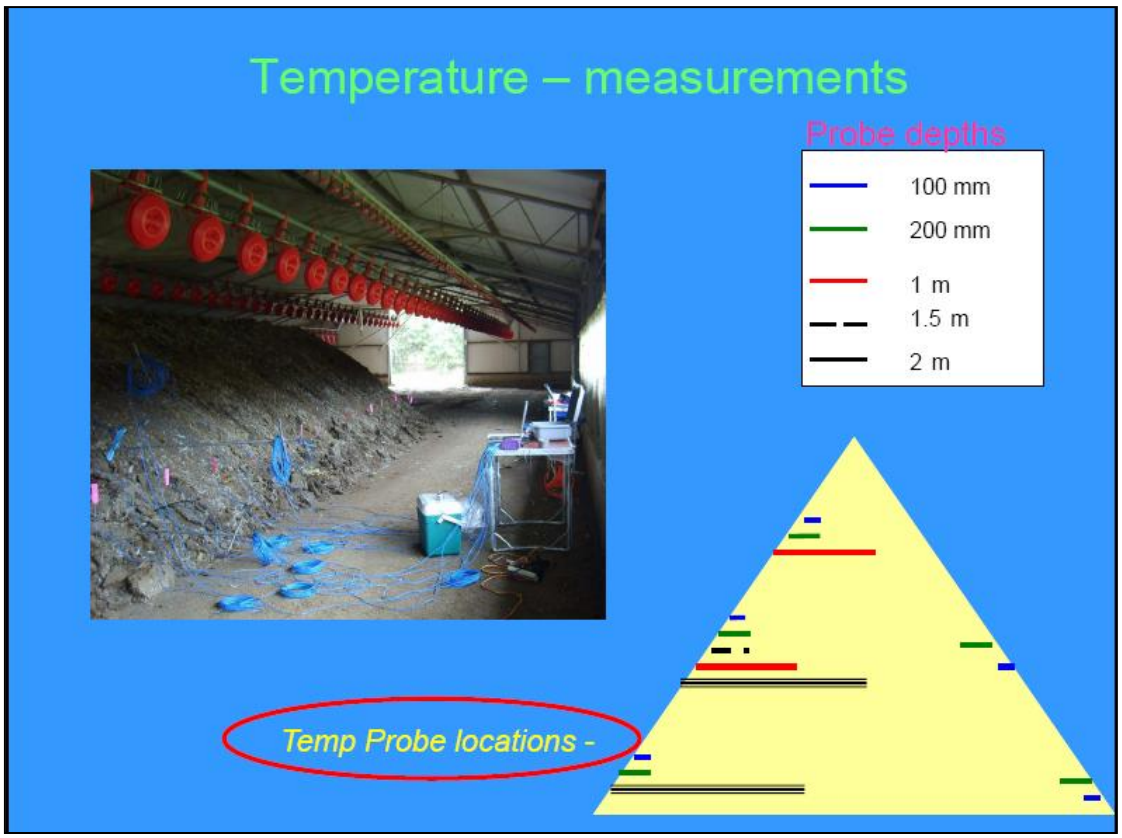
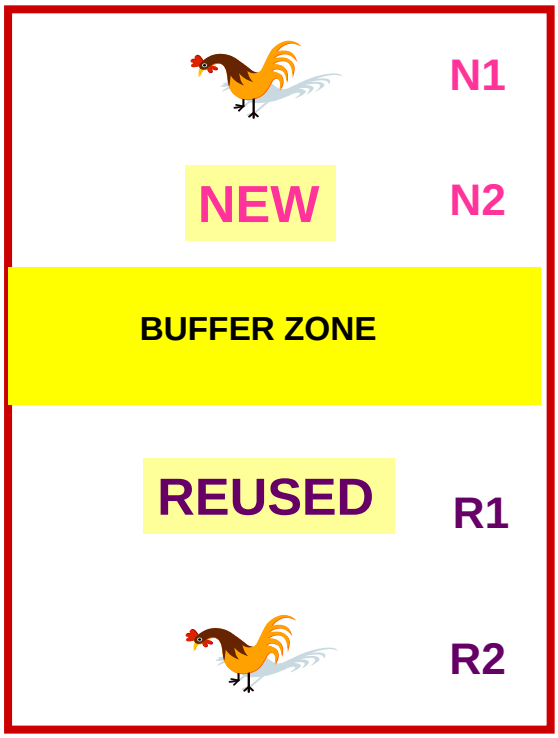


Figure 2.5 Illustration of sampling zones used when sampling spread litter during a chicken cycle.



Environmental samples

Intensive weekly environmental sampling was performed on the Donnybrook farm. This work involved samples collected late in one broiler cycle and then weekly across the next broiler cycle. As with the litter sampling described above, the shed was regarded as consisting of a new litter section (the brooder section), a buffer zone and a re-used litter section (the grow-out end) (see Fig 2.5). No sampling was done in the buffer zone.

In the study, two sheds were examined across the same time period. The sheds were visited on alternative weeks. Shed 1 had curtain side walls while Shed 6 had solid side walls.

Each week, four bays were randomly selected in both the brooder and grow-out end of the each shed. Dust from following surfaces was then sampled in of the selected bays:-

Drinker line
Feeder line
Feeder/Drinker Suspension Ropes
Bottom ledge of side wall
Top ledge of side wall
Surface of mini-vents in side wall
Surface of ceiling curtain
Surface of heater (brooder end only)
Surface of feeder pipe (grow-out end only)

The sampling was done using a sterile moistened transport swab. The swab was used to sample a 25 cm^2 in a zig-zag motion (both horizontally and vertically). The swab was then placed in transport media and transported on ice to the laboratory.

As well, the litter from the centre line of each of two bays was sampled as described previously. The litter was transported on ice to the laboratory.

Water activity

Water activity was tested for using a water activity meter (Water Activity- Decagon Safe Storage Quick Check). Water activity measurements were carried out on composite samples as soon as they were collected in the field. The probe was inserted into the sample bag after all air was eliminated and kept for about a minute until the readings were stable.

Moisture content

Once sampling preparation (i.e. compositing) was completed in the laboratory, a sub sample was drawn from the same quarter allocated for microbiological sampling. This sample was stored in a zip lock bag without headspace to minimise moisture loss and was stored at 4ºC for moisture analysis. The moisture content was determined as described in Chapter 1.

pH

The pH was measured using the composites used in the microbiological analysis. A 6 g sample of litter was mixed in 24 ml of distilled water and the pH recorded after standing for 5 min.

Shed relative humidity (RH)

This was determined using a Kestrel - Kestel 4000 Pocket Weather Tracker.

Microbiological analysis

All quantitative microbiological analysis was done using the methods described in Chapter 1. Additionally for *Salmonella* CHROMagar – Salmonella (Difco) was also plated in addition to XLD. All litter samples were also subjected to a presence/absence test for *Salmonella*. This presence/absence test was performed using 25 g of litter.

Some of the environmental samples, as detailed in the Results section, were subjected to a presence/absence test for *Salmonella*. For these environmental samples, the swab was placed in 10 ml of buffered peptone water which was then used for the enrichment step.

Selected *Bacillus* isolates were identified using a commercial kit (Bacillus Kit - API 5ºCH).

Results

Pile Studies

The details of the piles are listed in Table 2.1

Table 2.1 Physical Dimensions and other information for Litter Piles

Farm	General Details	Pile Dimensions	
Donnybrook cycle 1 **Pile duration** **6 days**	October 2006 First chicken cycle 39,400 chickens Final Pick Up 16/10/2006 Pushed Up 17/10/2006 Litter - Pine shavings Shed Moist	Pile 2 Height 1.7 m Length 9.8 m Depth 7.3 m Pile = litter with a single use	Pile 1 Height 1.87 m Length 14.5 m Depth 6.8 m Pile to be removed
Donnybrook cycle 2 **Pile duration** **5 days**	January 2007 Second chicken cycle ~40,000 chickens Final Pick Up 2/1/2007 Pushed Up 3/1/2007 Litter - Pine shavings	Termed Pile A Height 1.65 m Length 9 m Depth 4 m Pile = litter with a single use	Termed Pile B Height 1.1 m Length 7 m Depth 5 m Pile = litter with a single use
Laravale cycle 1 **Pile duration** **6 days**	November 2006 First chicken cycle ~ 32,5000 chickens Final Pick Up 6/11/2006 Pushed Up 10/11/2006 Litter - Pine shavings	Only one pile Height 2.2 m Length 17.2 m Depth 5.3 m Pile = litter with a single use	
Laravale cycle 2 **Pile duration** **4 days**	January 2007 Second chicken cycle ~ 32,5000 chickens Final Pick Up 23/1/2007 Pushed Up 24/1/2007 Litter - Pine shavings High ammonia levels in shed	Only one pile Height 2.1 m Length 28 m Depth 6 m Pile = litter from present and previous broiler cycle	
Laravale cycle 3 **Pile duration** **5 days**	April 2007 Third chicken cycle ~ 32,5000 chickens Final Pick Up 2.04.2007 Pushed Up 3.04.2007 Litter - Pine shavings	All old litter removed. This plie is singe use litter Height 2 m Length 8 m Depth 3.5 m Pile = litter with single use and multiuse from last time, i.e. cycles 1 & 2	

Shaded squares = piles used for trials

E. coli *die-off in piles through cycles 1 and 2 - Donnybrook*

Figures 2.6 and 2.7 illustrate the die-off of *E. coli* over two sequential litter cycles. In both cycles, the pile-up process reduced *E. coli* levels. In cycle 1, *E. coli* was present at around 10^8 CFU per g in litter under feeders and at around $10^{4.5}$ CFU per g in litter under drinkers (day 52). In the pile itself, the only sample positive for *E. coli* at day 6 was the Bottom Side B sample (Top samples, the other Bottom sample and the Core sample were below the detection limit). In the spread litter, prior to chicken entry, the *E. coli* levels were below the detection limit. In the second cycle, *E. coli* was detected in all locations on day 1. On day 3, *E. coli* was absent in one of the two Top samples, one of the two Bottom samples and the Core. *E. coli* was detected again in all locations except the Core on day 5, the last day of the pile (at levels 4 logs lower than in the litter prior to pick-up). The spread litter also yielded *E. coli* (at levels equivalent to that in the litter at pick up). However, this was probably due to the fact that chickens had already entered this section of the shed by the time of sampling. Overall, the levels of *E. coli* by the end of the pile up process were higher in cycle 2 than cycle 1.

Salmonella *die-off in piles through cycles 1 and 2 - Donnybrook*

Figures 2.8 and 2.9 illustrate the die-off of *Salmonella* over two litter sequential cycles. *Salmonella* was detected in the litter before bird pick up prior to litter cycle 1 (day 52 of the chicken cycle) at levels of around 100 MPN/g. However, *Salmonella* was not detected at any sampling site at any time in the litter pile in cycle 1 or in the spread litter prior to chicken placement (day 2 of the next chicken cycle) (both by MPN testing and presence/absence testing of 25 g samples). In cycle 2, *Salmonella* was again present in the litter prior to the final pick-up at levels of around 100 MPN/g. *Salmonella* was detected in the early stages of the pile-up process (both Bottom samples were positive at day 1 of the pile) but no sample in the litter pile was positive at day 5. The spread litter was positive at around 100 MPN/g, probably due to the presence of young chicks on the litter (sample taken at day 17 of the chicken cycle and chickens were already present). No carry over of *Salmonella* from the piled litter to the subsequently spread litter appears to have occurred.

Campylobacter *die-off in piles through cycles 1 and 2 - Donnybrook*

Figures 2.10 and 2.11 illustrate the die-off of *Campylobacter* over two sequential litter cycles. *Campylobacter* was present in the litter prior to final bird pick up in both cycles with the levels reaching 10^6 MPN/g (much higher than the *Salmonella* levels reported above). However, *Campylobacter* was not detected at any time point in any pile position in both litter cycles 1 and 2. For both cycles, the spread litter was negative for *Campylobacter* – even the sample collected after entry of chickens onto the litter.

Bacillus *die-off in piles through cycles 1 and 2 – Donnybrook*

Figures 2.12 and 2.13 illustrate the levels of *Bacillus* spp. over two sequential cycles. *Bacillus* spp. were absent in the litter at the end of chicken cycle 1 (day 57), a cycle that had commenced after a full cleanout. *Bacillus* species were detected at all positions and all times through the pile-up process. The spread litter was positive for *Bacillus* spp after the pile-up process. *Bacillus* continued to be present in the litter, being detected in two of the litter samples at levels of between 10^2 to around 10^6 CFU per g of litter prior to pick up in chicken cycle 2. Again, *Bacillus* spp. and were detected at all positions and all time points in the pile and are also present in the spread litter. The main species were *B. mycoides* and *B. cereus* (Table 2.2).

Figure 2.6 *E. coli* levels in litter at feeder and drinker (before and after litter piling) and in different positions in the litter pile over time (Donnybrook, litter cycle 1, October 2006). Chicken age when the litter was collected is shown in red font. There are three sampling days per pile position (with an absence of a bar indicating a level below the indicated detection level).

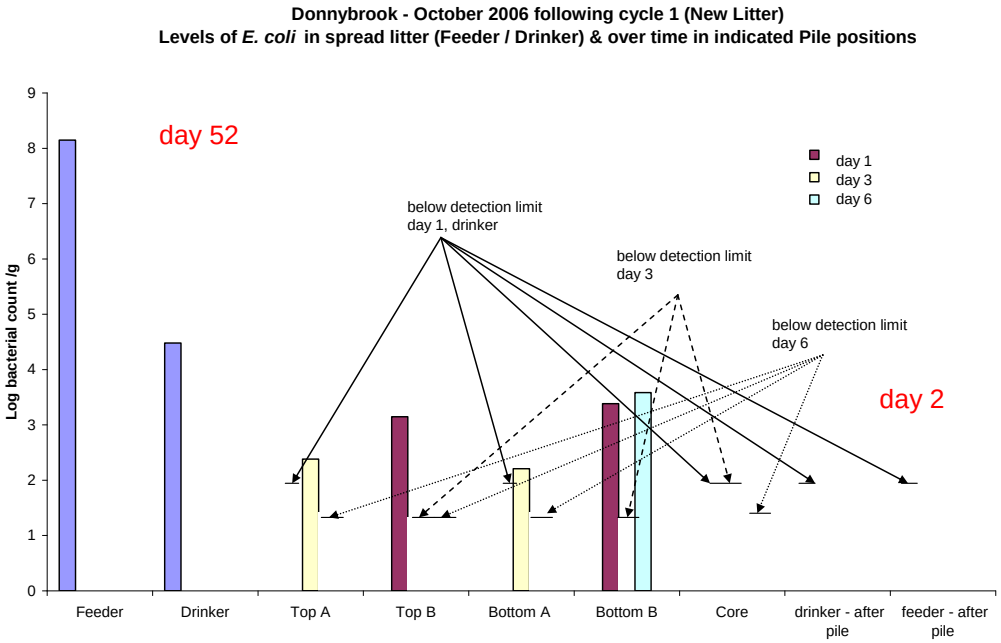


Figure 2.7 *E. coli* levels in litter at feeder and drinker (before and after litter piling) and in different positions in the litter pile over time (Donnybrook, litter cycle 2 January 2007)**.** Chicken age when the litter was collected is shown in red font. There are three sampling days per pile position (with an absence of a bar indicating a level below the indicated detection level).

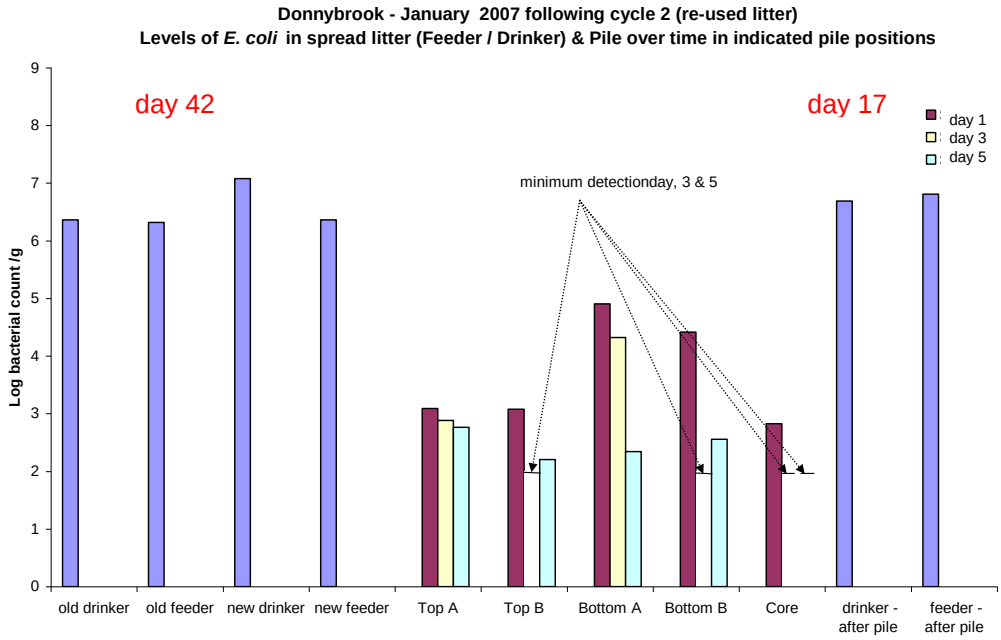


Figure 2.8 *Salmonella* levels in litter at feeder and drinker (before and after litter piling) and in different positions in the litter pile over time (Donnybrook, litter cycle 1 October 2006). Chicken age when the litter was collected is shown in red font. There are three sampling days per pile position (with an absence of a bar indicating a level below the indicated detection level).

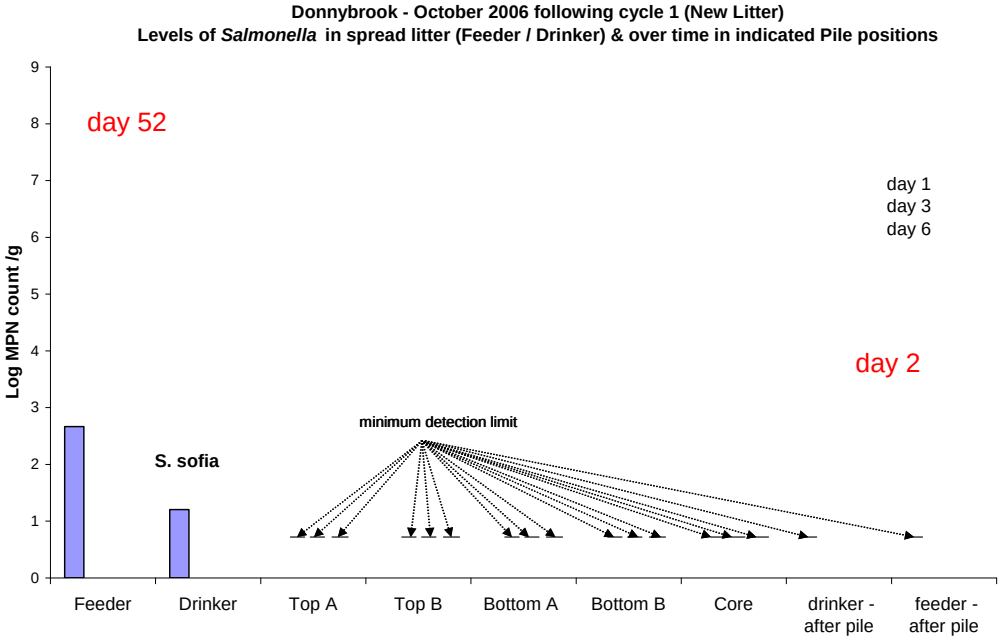


Figure 2.9 *Salmonella* levels in litter at feeder and drinker (before and after litter piling) and in different positions in the litter pile over time (Donnybrook, litter cycle 2, January 2007)**.** chicken age when the litter was collected is shown in red font. There are three sampling days per pile position (with an absence of a bar indicating a level below the indicated detection level).

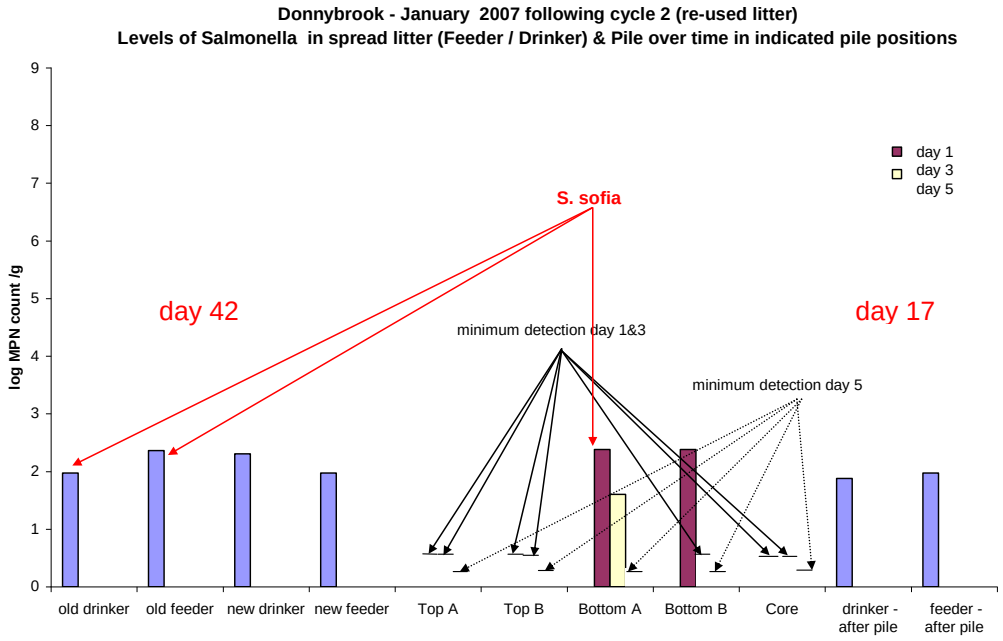


Figure 2.10 *Campylobacter* levels in litter at feeder and drinker (before and after litter piling) and in different positions in the litter pile over time (Donnybrook, litter cycle 1, October 2006). Chicken age when the litter was collected is shown in red font. There are three sampling days per pile position (with an absence of a bar indicating a level below the indicated detection level).

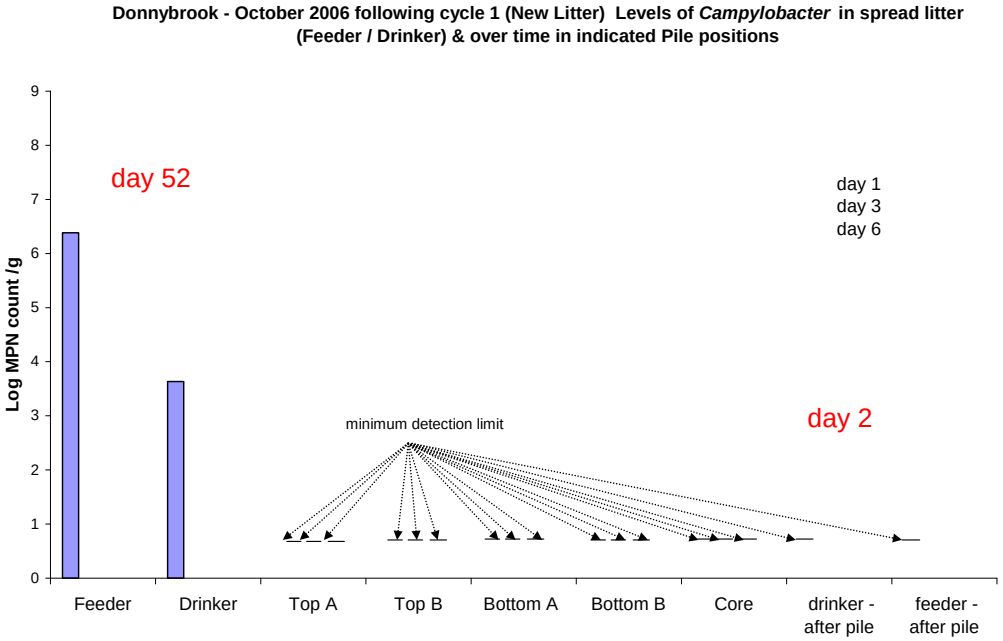


Figure 2.11 *Campylobacter* levels in litter at feeder and drinker (before and after litter piling) and in different positions in the litter pile over time (Donnybrook, litter cycle 2, January 2007). Chicken age when the litter was collected is shown in red font. There are three sampling days per pile position (with an absence of a bar indicating a level below the indicated detection level).

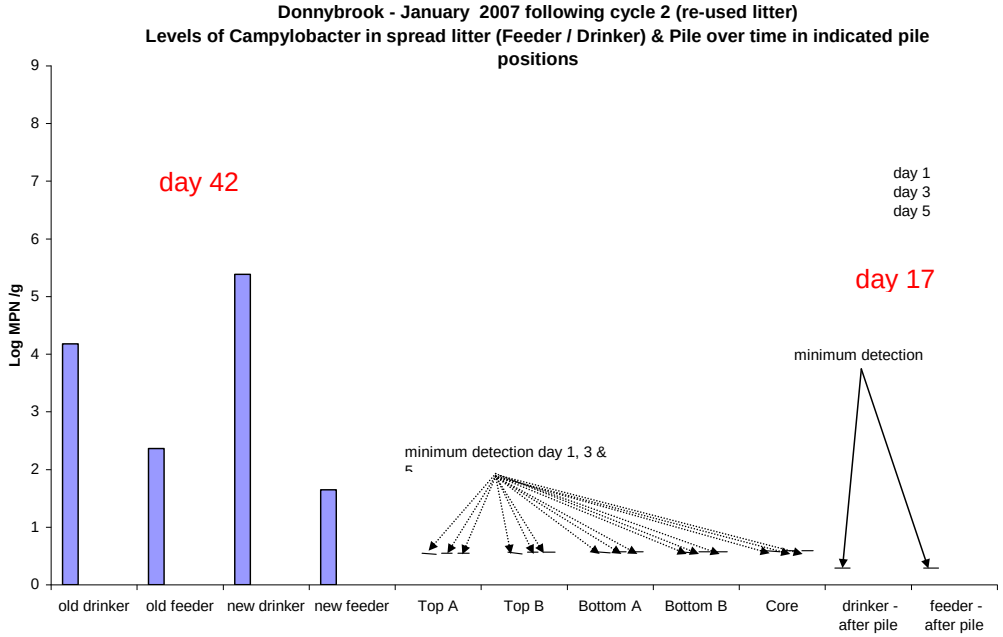


Figure 2.12 *Bacillus* levels in litter at feeder and drinker (before and after litter piling) and in different positions in the litter pile over time (Donnybrook, litter cycle 1, October 2006). Chicken age when the litter was collected is shown in red font. There are three sampling days per pile position (with an absence of a bar indicating a level below the indicated detection level).

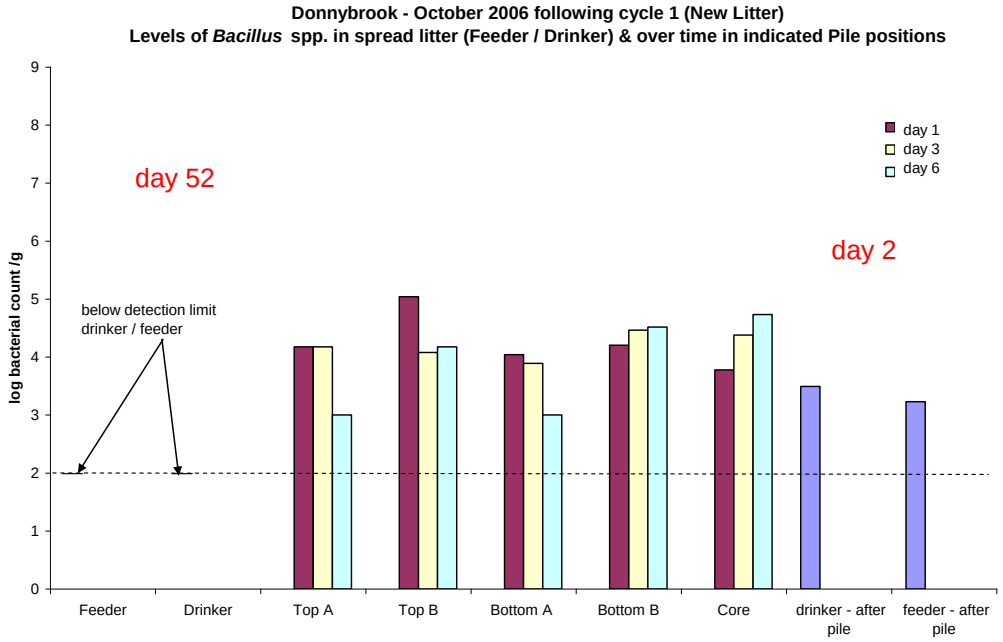


Figure 2.13 *Bacillus* levels in litter at feeder and drinker (before and after litter piling) and in different positions in the litter pile over time (Donnybrook, litter cycle 2, January 2007)**.** Chicken age when the litter was collected is shown in red font. There are three sampling days per pile position (with an absence of a bar indicating a level below the indicated detection level).

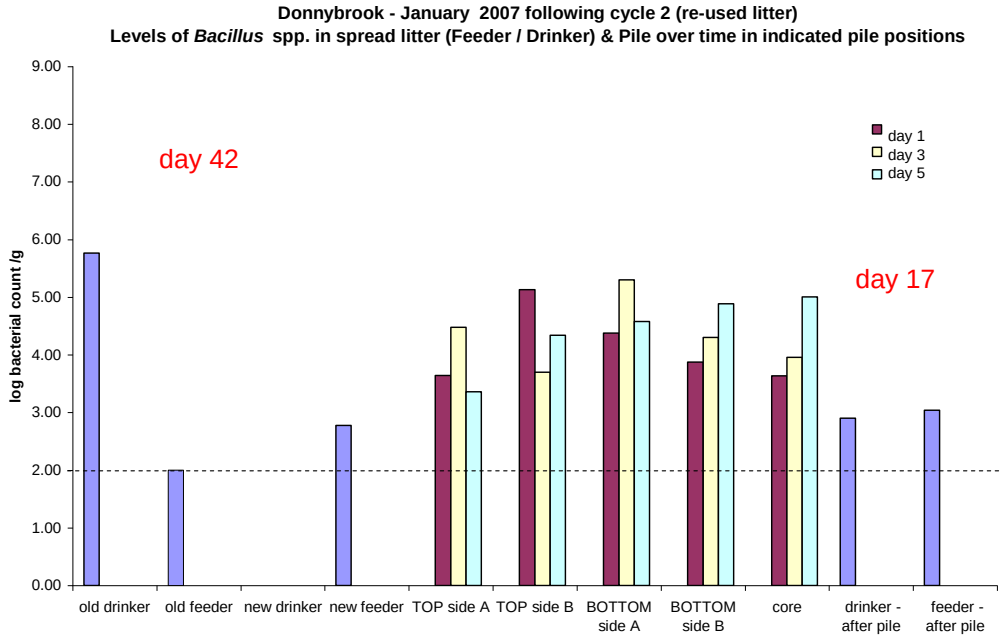


Table 2.2 Identity of *Bacillus* spp. in litter from Donnybrook

Farm (Cycle)	Day of Pile	Position	Identification (Number of isolates)
Donnybrook (chicken cycle 1)	1	Top B Side	*B. mycoides* (2)
	1	Bottom B Side	*Geobacillus stearothermophilus* (1)
	5	Top B Side	*B. cereus* (1)
			B. mycoides (2)
Donnybrook (chicken cycle 2)	End of cycle – prior to piling	Spread litter	*B. cereus* (3)

pH in piles through cycles 1 and 2 – Donnybrook

Figure 2.14 and 2.15 illustrate the pH levels before pick-up, through the litter pile and in the spread litter over two litter cycles. In cycle 1, the litter at the end of the chicken cycle had a pH of 8.8. In the pile, the pH was in the range of 8.5 to 9.0 for all positions expect the Core. In the Core samples, there was a consistent fall in pH from around 9.0 to below 8.0. The spread litter had a pH of around 8.0, an overall drop in pH. In litter cycle 2, the spread litter prior to piling, the litter pile (all positions, all times) and the spread litter were all around pH 8.5

aW in piles through cycles 1 and 2 – Donnybrook

Figures 2.16 and 2.17 illustrate water activity (aW) levels before pick-up, through the litter pile and in the spread litter over two cycles. The vertical axis is divided into three zones, shown as green, black and red. These zones represent aW levels associated with the probability of isolating *Salmonella* in broiler litter as suggested by Carr *et al.* (1994)

aW range 0.75-0.83 – Negative (Green zone)
aW range 0.83-0.90 – Transition zone (Black zone)
aW range 0.90-0.96 – Positive (Red zone)

A further study (Payne et al. 2007) has observed that the aW range 0.95- 1.00 is not associated with high *Salmonella* populations.

For litter cycle 1 almost all water activity levels recorded at all pile positions and times were around 1 or greater (Figure 2.16). This suggests that the physical conditions of the pile tended to trap water vapours within the pile, ensuring a humid environment.

For litter cycle 2, similar high water activities were observed again in all pile locations excepting Bottom side A (Figure 2.17). This site had a mean water activity of 0.97 (values of 0.91, 0.94, 0.99, 0.99 and 1.04) on day 1 of the pile, a similar level at day 3 and an even lower level of 0.9 by day 5 of the pile. Interestingly, this site of relatively lower water activity was positive for *Salmonella* at around 10^2 MPN/g on days 1 and 3, although negative at day 5. In contrast, the Bottom B site at day 1 of the pile, which had a high water activity of 1.08, was also positive for *Salmonella*.

In the spread litter prior to the pile for litter cycle 2, there levels of *Salmonella* of around 100 MPN/g litter around the drinker and feeder regions (Figure 2.9). These regions had relatively low water activity levels of between 0.87 – 0.90 (Figure 2.17). In the spread litter after the second litter pile, the water activities in the grow-out end were quite low (0.8 and 0.85), although *Salmonella* was present at levels around 100 MPN/g (Figures 2.9, 2.17).

Overall the water activities of the of the spread litter following pile-up was lower than when in the pile.

Relationship between aW and moisture in piles through cycles 1 and 2 – Donnybrook

There does not seem to be a linear relationship between aW and moisture, in either pile samples or spread litter (Figures 2.18 and 2.19). This is most evident in the spread litter prior to the pile up process in cycle 2 (Figure 2.19). These litter samples (collected at Day 42 of the chicken cycle) all had water activities in a tight range (0.87-0.90) yet the moisture content varied from 25 to 45%. In contrast during litter cycle 2 (Figure 2.19) the Top B samples were relatively dry (moisture content 24 – 27%) but had high water activities (1.01 – 1.02). This phenomenon of non-linear relationship is observed through various pile locations.

The other observation from both cycles is that in spread litter the water activity curves are below the moisture content curves while in the litter pile, the water activity curves are above the moisture content curves. This emphasises the point made previously – the nature of the litter pile appears to trap water vapours in the pile.

Figure 2.14 pH levels in litter at feeder and drinker (before and after litter piling) and in different positions in the litter pile over time (Donnybrook, litter cycle 1, October 2006). Chicken age when the spread litter was sampled is shown in red font. There are three sampling days per pile position (day 1, day 3, day 6).

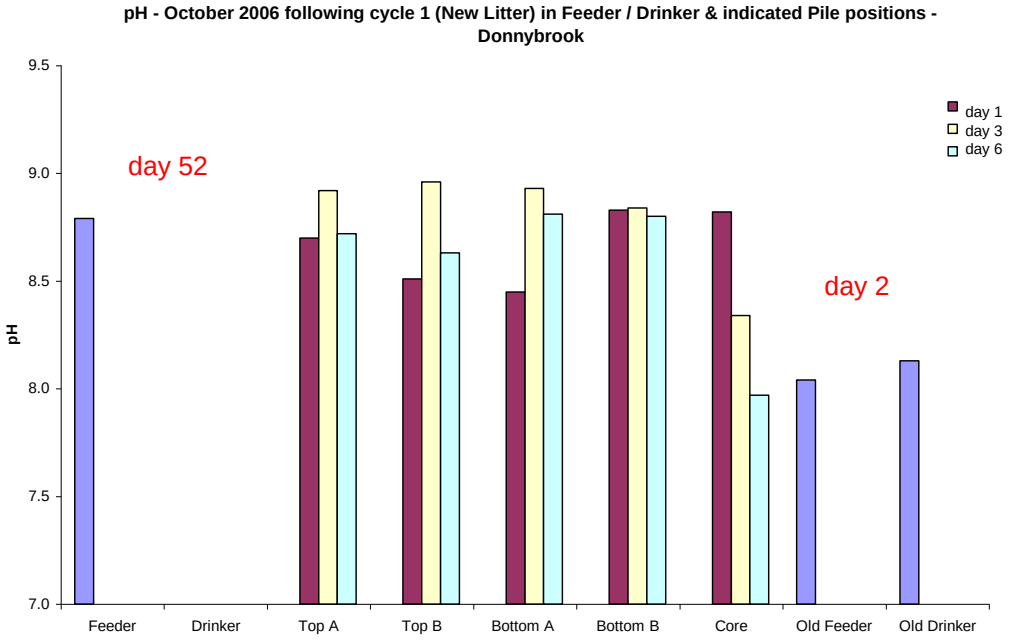


Figure 2.15 Figure pH levels in litter at feeder and drinker (before and after litter piling) and in different positions in the litter pile over time (Donnybrook, litter cycle 2 January 2007). Chicken age when the spread litter was sampled is shown in red font. There are three sampling days per pile position (day 1, day 3, day 5).

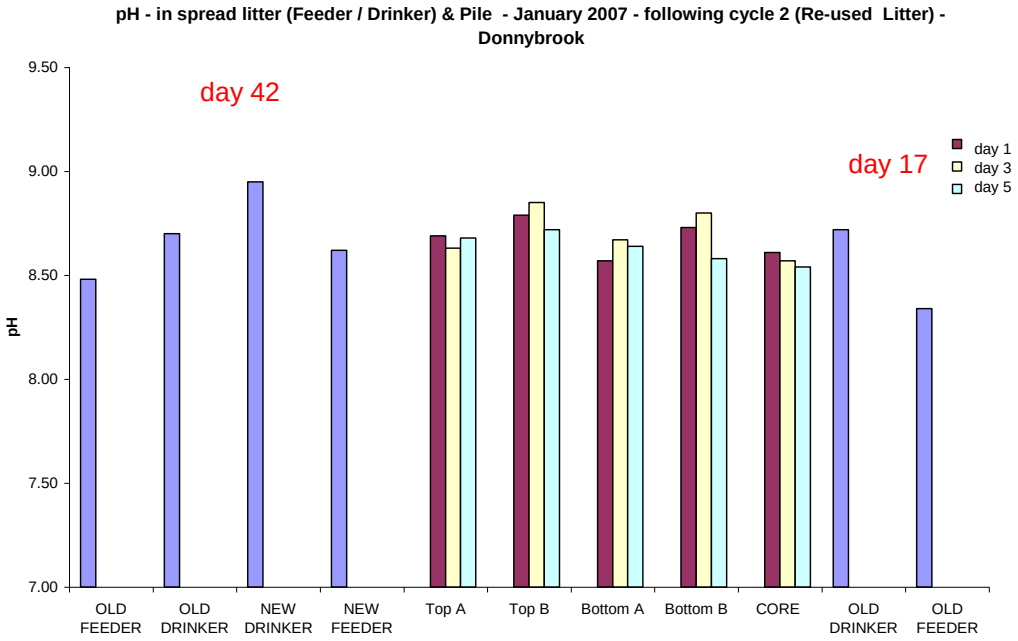


Figure 2.16 Water activity levels in litter at feeder and drinker (before and after litter piling) and in different positions in the litter pile over time (Donnybrook, litter cycle 1, October 2006). Chicken age when the spread litter was sampled is shown in red font. There are three sampling days per pile position (day 1, day 3, day 6). The relative humidity of the shed is shown in blue font.

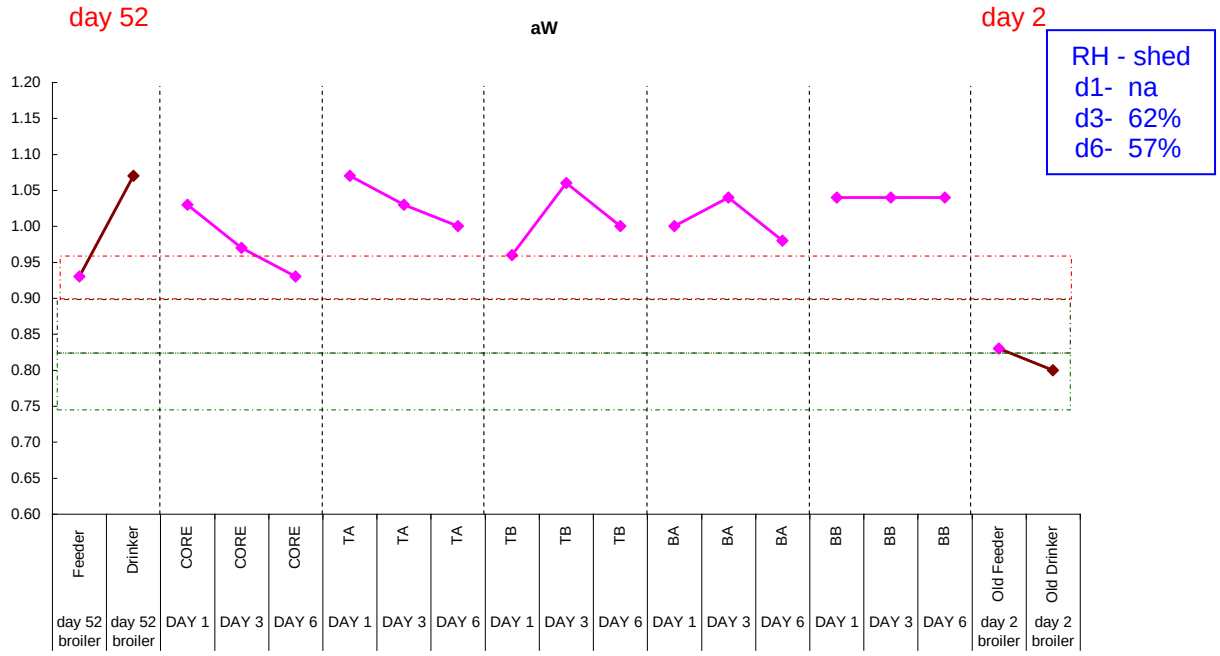


Figure 2.17 Water activity levels in litter at feeder and drinker (before and after litter piling) and in different positions in the litter pile over time (Donnybrook, littercycle 2 January 2007)**.** Chicken age when the spread litter was sampled is shown in red font. There are three sampling days per pile position (day 1, day 3, day 5). The relative humidity of the shed is shown in blue font.

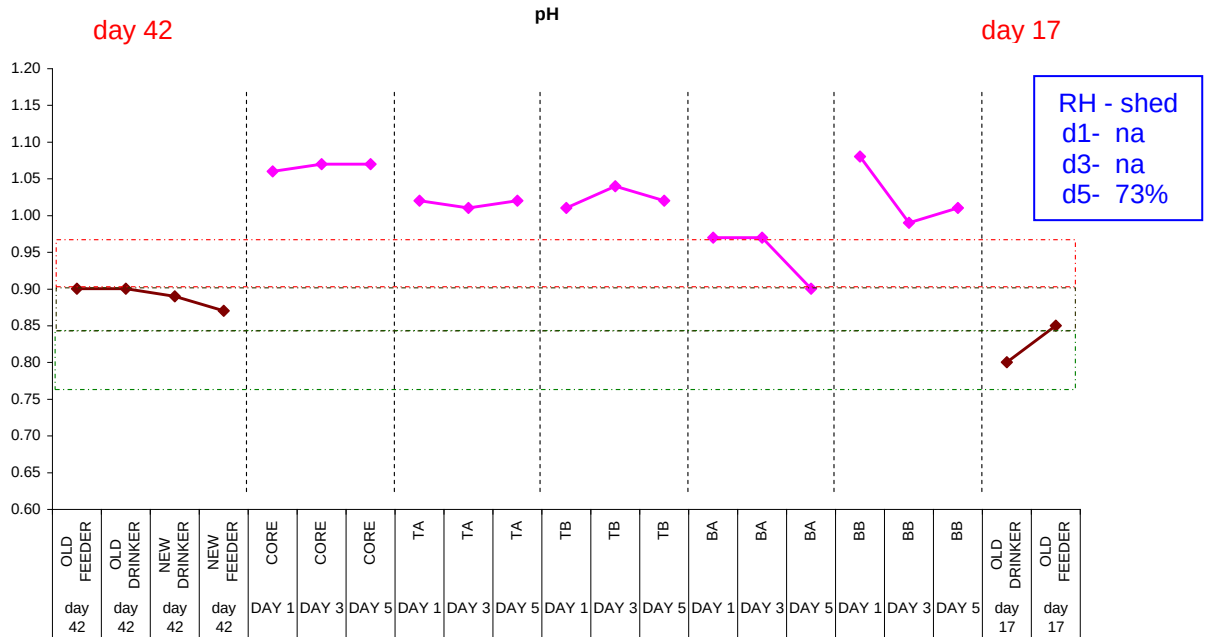


Figure 2.18 % Moisture levels and water activity in litter at feeder and drinker (before and after litter piling) and in different positions in the litter pile over time (Donnybrook, litter cycle 1, October 2006).

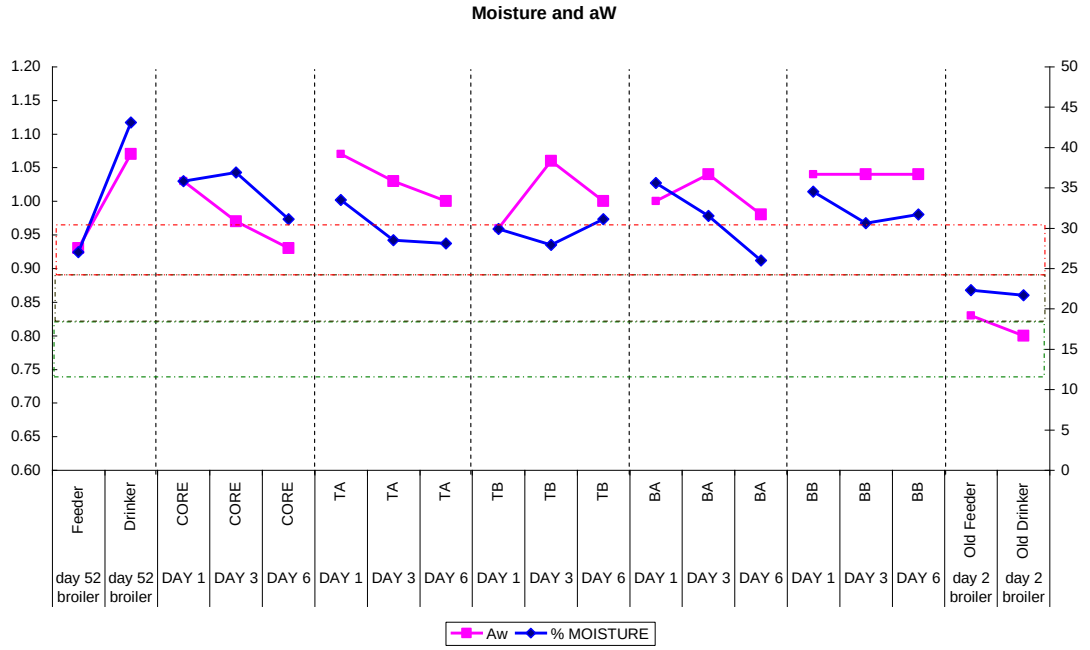
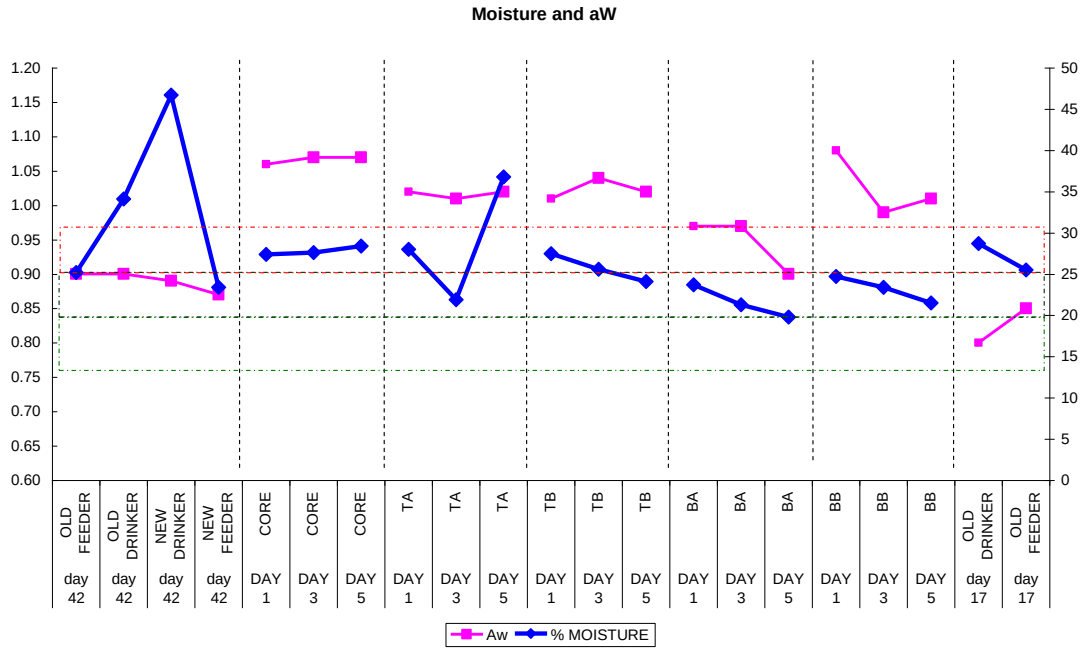


Figure 2.19 % Moisture levels and water activity in litter at feeder and drinker (before and after litter piling) and in different positions in the litter pile over time (Donnybrook, litter cycle 2, January 2007)



Relationship between temperatures at different pile locations in cycles 1 and 2 – Donnybrook

Figures 2.20 and 2.21 present the temperature profiles at different pile locations over the life of the relevant pile for two sequential litter cycles. In each graph, the depth of the temperature measurement (1 m, 100 mm, 200 mm and so on), the side of the pile (A or B) (for the surface measurements) and the location in terms of the slope profile of the pile (bottom of the pile, middle of the pile and top of the pile) are shown where relevant. Both piles had similar physical dimensions (see Table 2.1).

Top measurements

The heating profile in both cycles was very similar at this site. Surface temperatures were higher (~ 65^{o}C) at 200 mm depth compared to 100 mm depth (50 - 55^{o}C) in both cycles.

Middle measurements

In cycle 1 the temperature was consistently higher for the probe at 200 mm as compared with the probe at 100 mm depth. However, at the 450 mm depth, the temperature rise took longer to occur. As well, at 100 mm depth, while the maximum temperature (around 62^{o}C) was rapidly reached, there was a gradual temperature drop from day 2 of the pile onwards. Overall, depth mainly dictated the temperatures reached and the stability of such temperatures during cycle 1.

In contrast during cycle 2 the 100 mm probe never increased above temperatures of around 40ºC - 45ºC. The matching 200 mm probe on the same side of the pile ranged from around 50ºC - 55ºC while the 200 mm probe on the other side of the pile (side A) reached temperatures around 57ºC - 65ºC. Unlike cycle 1, all these locations maintained fairly stable temperatures.

Bottom

During cycle 1 the temperatures at the 100 and 200 mm depths were somewhat higher on side B of the pile (45ºC - 53ºC at 100 mm and 53ºC - 55ºC at 200 mm) as compared to side A (53ºC - 55ºC at 100 mm and 58^{o}C - 60^{o}C at 200mm). Similarly in cycle 2, there was a difference between the two sides of the pile. These side differences may have something to do with the cooling effect due to the wind direction on the pile from open panels on the sides of the shed.

As noted in the middle measurements, the cycle 2 bottom measurements were noticeably lower than the temperatures reached in cycle 1. The over all maximum was around 50ºC at a depth 200 mm (for a brief period).

Core

In cycle 1 core measures were taken at a depth of 1 m and 2 m at the middle (slope face) of the pile. In cycle 2, probes were set at 1 m, 1.5 m and 2.0 m depth at around 200 mm vertical height from the bottom of the pile. A 1.5 m probe was inserted in the middle (slope face) of the pile. As well, a 0.5 m deep probe was inserted towards the top of the slope face of the pile.

In both cycles, the cores took longer to heat up than surface and sub-surface samples. In addition, the bottom core in cycle 2 samples showed a slower temperature rise than core samples in the middle (slope face) of the pile.

During cycle 1 the 1 m probe showed a gradual increase over the 6 days to reach a temperature of around 65ºC. For the 2 m probe, there was a gradual increase, although the temperature was always lower than the 1 m probe and a lower maximum temperature (55ºC) was reached.

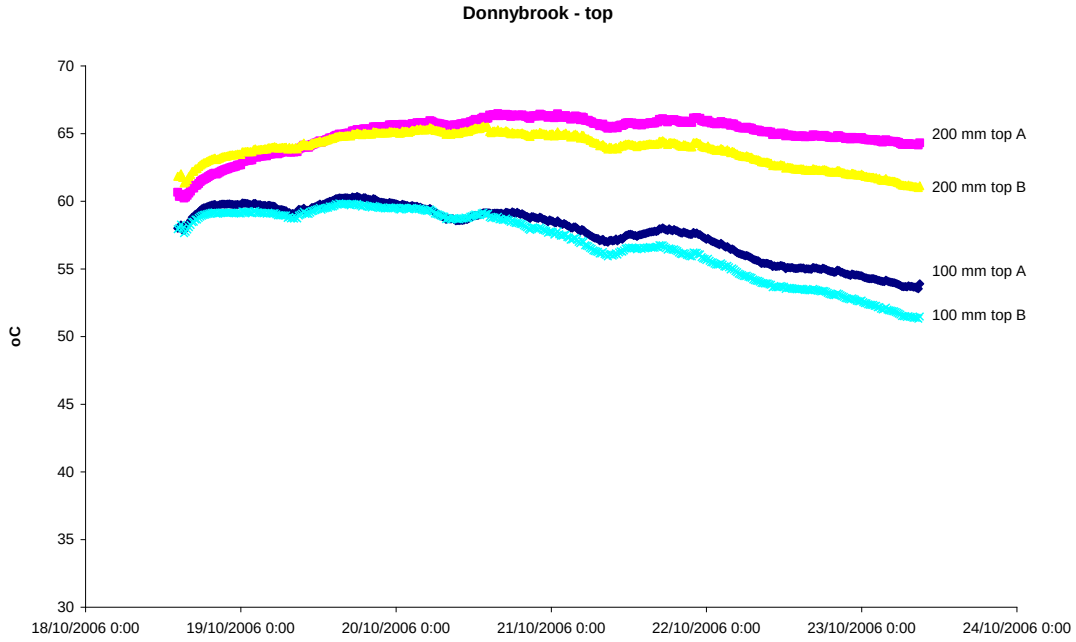
In cycle 2, the probes at 0.5 m (near the top of the slope face) and 1.5 m (placed in the middle of the slope face) did reach 65ºC. These high temperatures were however not reached at the depths of 1.5, 1.0 and 2 m in the bottom of the pile. Indeed, at these depths, stable temperatures of around 33º - 40ºC were recorded over the life of the pile.

Overall

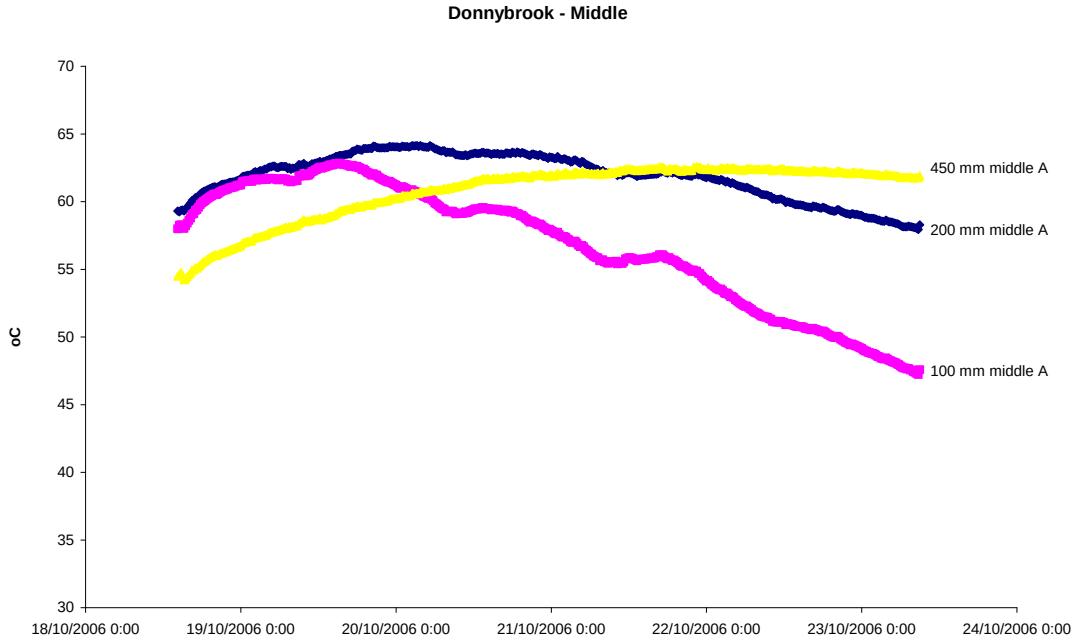
Across both cycles varying temperatures were reached across different pile locations. A common feature of both piles was that high surface temperatures were reached at the top of the pile. Temperatures recorded near the bottom of the pile (either at the surface or at depths) were lower than similar locations higher up in the pile. The temperatures at depth were often stable or slower in rising than the surface temperatures. The temperatures at depth did reach high levels but only in the middle or upper sections of the pile. The differences between the top and the bottom of the pile (at both surface and depth) could be influenced by both the insulation effect of the shed roof and by the cooling effect of the earth floor as well as the size of the pile.

Figure 2.20 Temperature profiles within litter pile over time (Donnybrook, litter cycle 1 October 2006. For each location, the depths at which the temperatures were recorded are shown. A) Near the top of the slope face of the pile; B) Middle of the slope face of the pile; C) Near the bottom of the slope face of the pile D) Core of the pile.

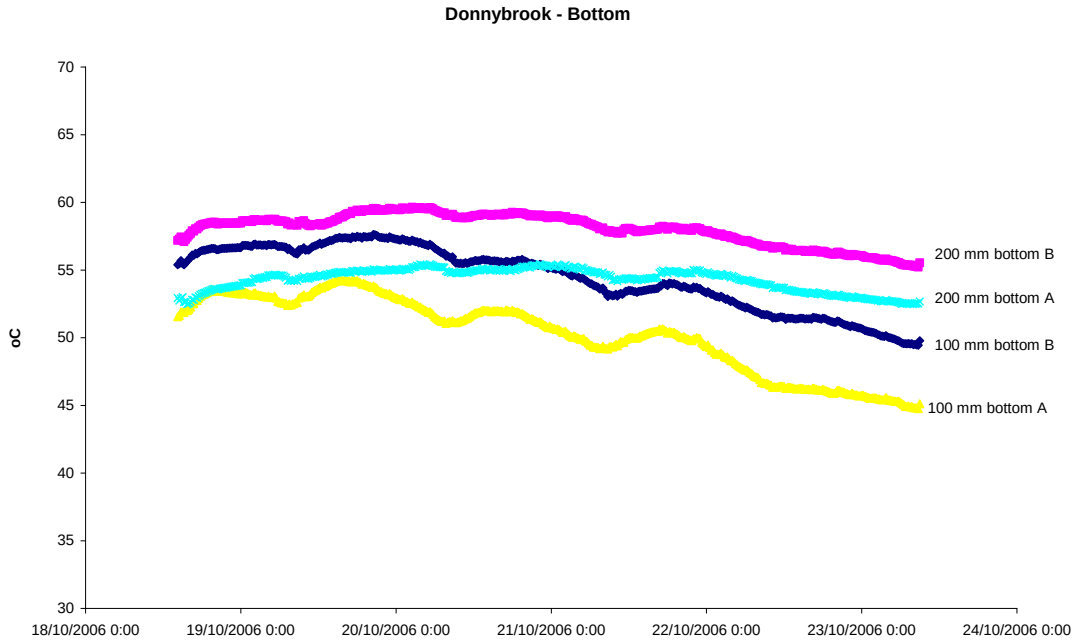
A.



B.



C.


Donnybrook - Bottom
70
65
60
55
50
45
40
35
30
oC
18/10/2006 0:00
19/10/2006 0:00
20/10/2006 0:00
21/10/2006 0:00
22/10/2006 0:00
23/10/2006 0:00
24/10/2006 0:00
200 mm bottom B
200 mm bottom A
100 mm bottom B
100 mm bottom A

D.

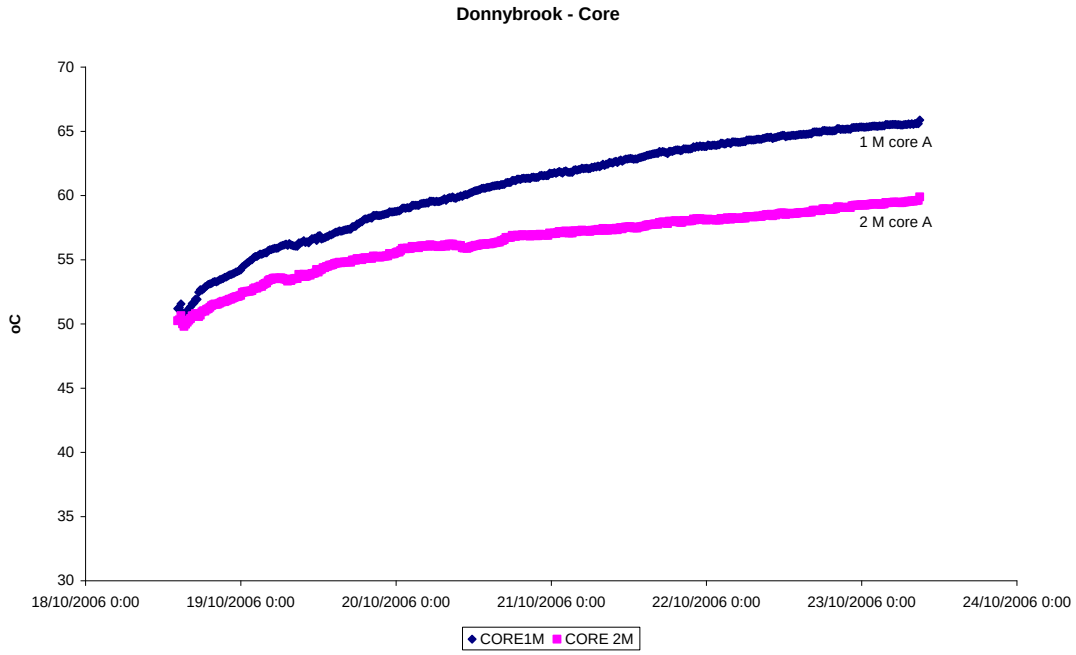
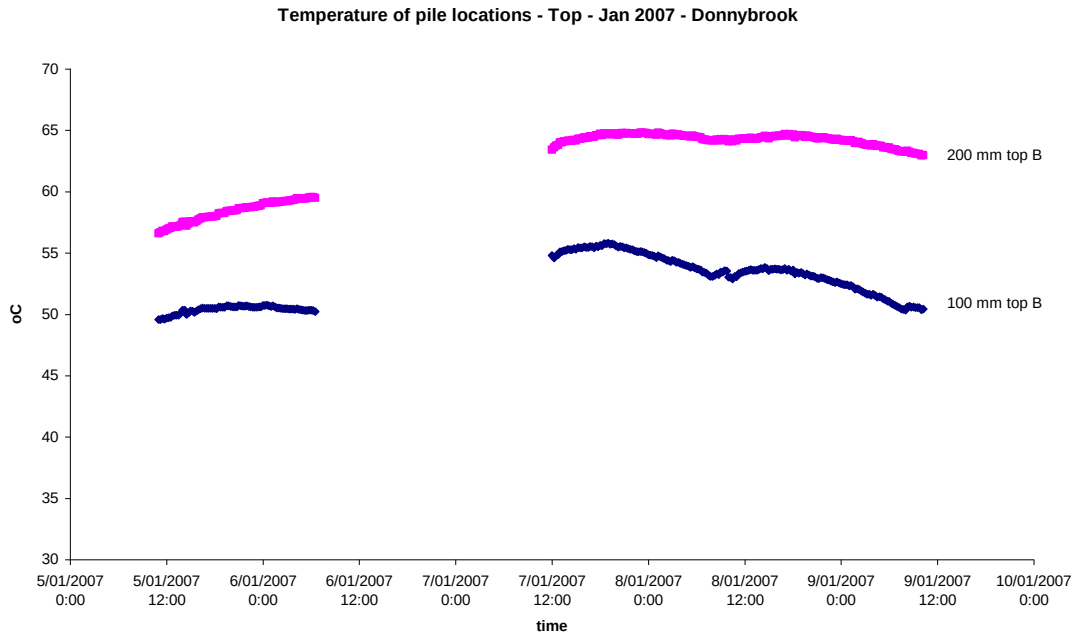
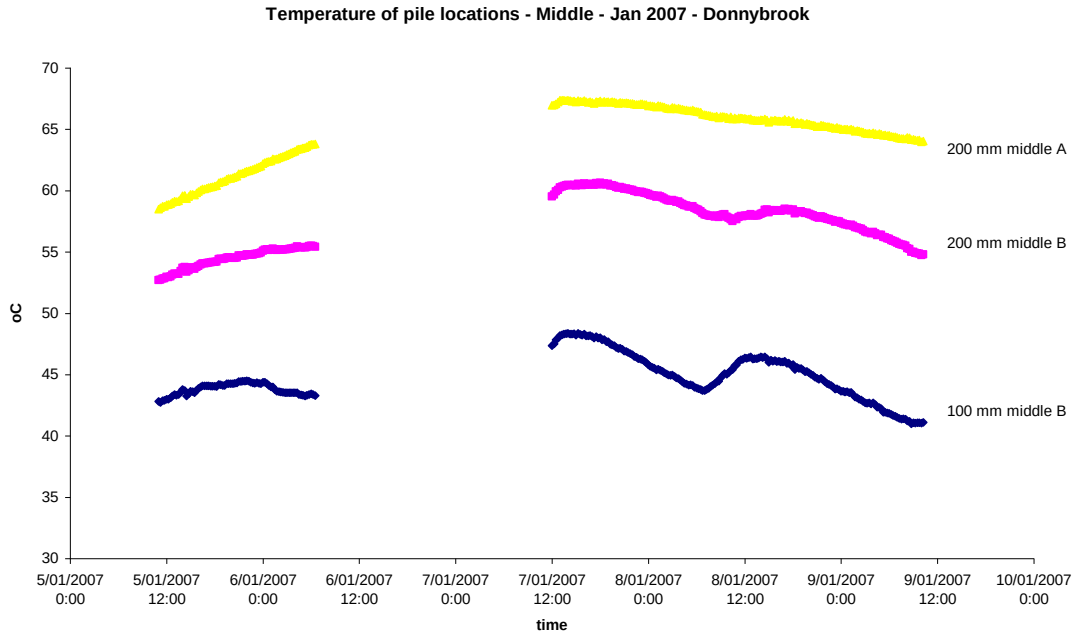

Donnybrook - Core
70
65
60
55
50
45
40
35
30
oC
18/10/2006 0:00
19/10/2006 0:00
20/10/2006 0:00
21/10/2006 0:00
22/10/2006 0:00
23/10/2006 0:00
24/10/2006 0:00
1 M core A
2 M core A
CORE1M
CORE 2M

Figure 2.21 Temperature profiles within litter pile over time (Donnybrook, litter cycle 2, January 2007). For each location, the depths at which the temperatures were recorded are shown. For a 24 h period data was lost, explaining the blanks in all lines A) Near the top of the slope face of the pile; B) Middle of the slope face of the pile; C) Near the bottom of the slope face of the pile D) Core of the pile.

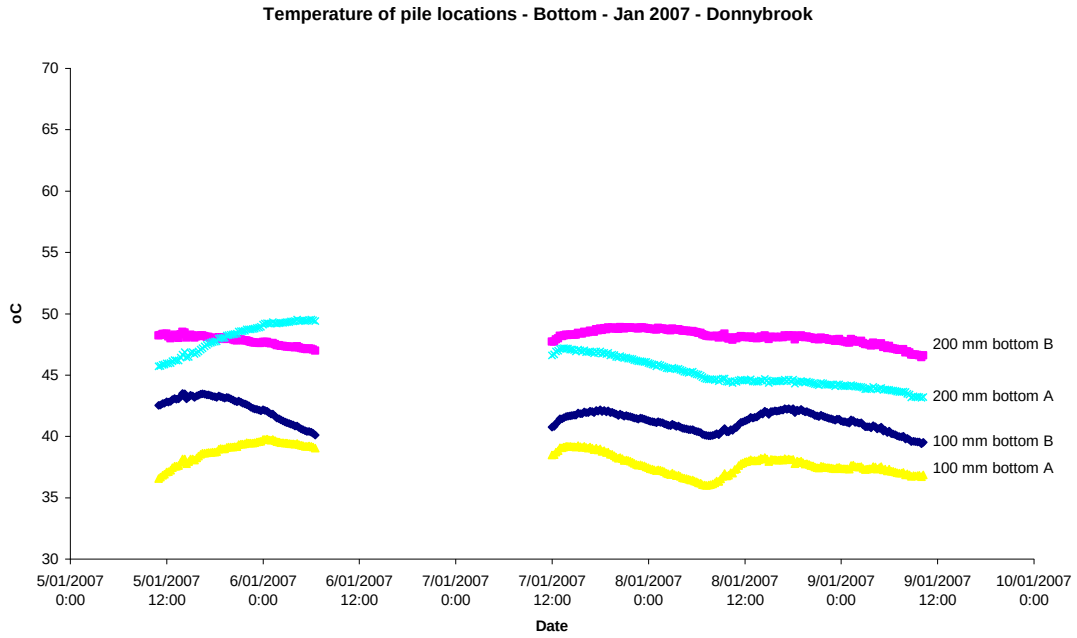
A.



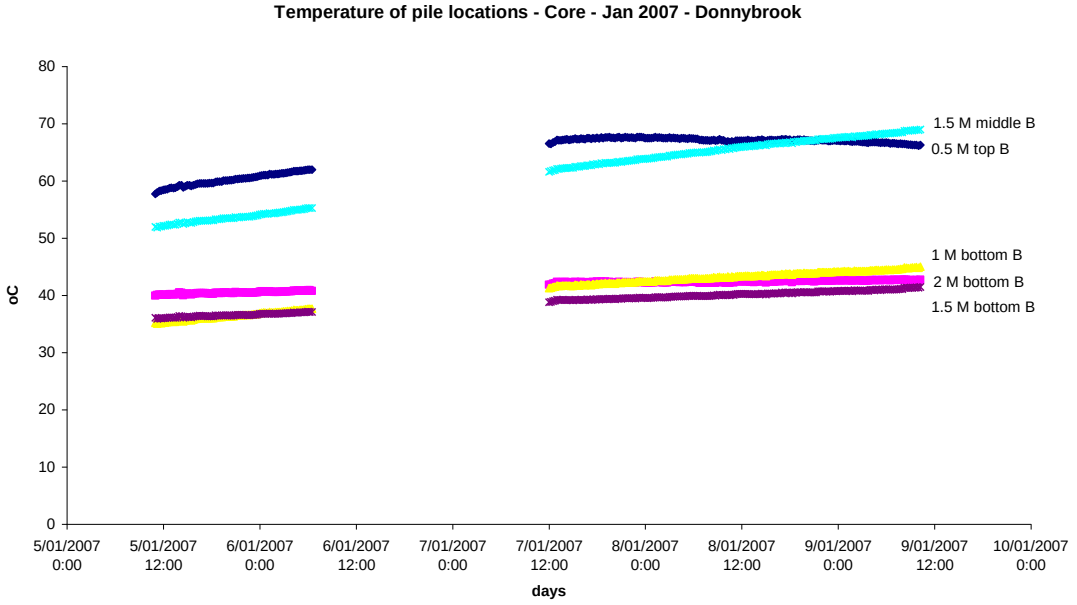
B.



C.


Temperature of pile locations - Bottom - Jan 2007 - Donnybrook
oC
70
65
60
55
50
45
40
35
30
5/01/2007 0:00
5/01/2007 12:00
6/01/2007 0:00
6/01/2007 12:00
7/01/2007 0:00
7/01/2007 12:00
8/01/2007 0:00
8/01/2007 12:00
9/01/2007 0:00
9/01/2007 12:00
10/01/2007 0:00
Date
200 mm bottom B
200 mm bottom A
100 mm bottom B
100 mm bottom A

D.


Temperature of pile locations - Core - Jan 2007 - Donnybrook
oC
80
70
60
50
40
30
20
10
0
5/01/2007 0:00
5/01/2007 12:00
6/01/2007 0:00
6/01/2007 12:00
7/01/2007 0:00
7/01/2007 12:00
8/01/2007 0:00
8/01/2007 12:00
9/01/2007 0:00
9/01/2007 12:00
10/01/2007 0:00
days
1.5 M middle B
0.5 M top B
1 M bottom B
2 M bottom B
1.5 M bottom B

E. coli die-off in piles through cycles 1, 2 and 3 - Laravale

Figures 2.22 to 2.24 illustrate the levels of *E. coli* in three litter cycles at Laravale. The levels in the litter prior to the pile-up process in the three cycles were typically around 10^6 CFU /g. On the first sampling day of all three piles (either day 0 for cycles 1 and 2 or day 1 for cycles 3), *E. coli* was still present in all pile locations in all three trials with the exception of the sub-core sample in cycle 2. The *E. coli* levels in the piles at this first sampling date ranged from 10^2 (the day 1 sampling) to 10^6 (the day 0 sampling) CFU/g. The pile age presumably explains the difference in these counts.

At the second sampling date, *E. coli* was still present in all pile locations except in the core and sub core of cycle 3 litter pile. However on the third day of testing of piles in all three pile cycles (i.e. days 6, 4 and 5 of pile life respectively) *E. coli* was absent in all pile locations.

Once the litter was spread no carry over was observed after litter cycle 1 – the spread litter was negative for *E. coli*. *E. coli* was not detectable on the last day of the litter pile for cycles 2 and 3, suggesting a die-off of *E. coli*. However, for these two cycles, *E. coli* was present at levels of $10^6 – 10^7$ CFU/g in the spread litter. These counts of *E. coli* are probably due to the presence of young chickens which had already entered this region of the shed when the litter was tested on days 13 and 17 of the subsequent chicken cycle.

Salmonella die-off in piles through cycles 1, 2 and 3 - Laravale

Figures 2.25 to 2.27 illustrate the levels of *Salmonella* in three litter cycles at Laravale.

For the cycle 1 study (Figure 2.25), *Salmonella* was only detected at a very low level in litter around the vicinity of the drinker on day 49 of the chicken cycle. This low and inconsistent presence of *Salmonella* is in marked contrast to the situation for *E. coli* described above.

In litter push-up cycle 1, the only sample yielding *Salmonella* was the Bottom side B sample on the first sampling day with only around 100 MPN/g being present. *Salmonella* was absent in all other pile locations on all sampling days (by both MPN and presence/absence tests). There was no carry over to the spread litter which was negative in both the MPN and presence/absence tests.

In litter push-up cycle 2, an interesting result was obtained. Even though *Salmonella* was absent on day 48 of the chicken cycle in all 4 locations tested (composite samples were created from multiple samples at each location), *Salmonella* was present at levels of 130 and 1,100 MPN/g at Bottom pile locations on sides A and B on the first day of pile sampling. On the second sampling day (Day 2 of the pile), *Salmonella* was present at 16 to >23 MPN/g at pile locations Top B, Core and Sub-core sites, all of which were negative for *Salmonella* on the first day of sampling. The >23 MPN/g result, which occurred at two spots (Top B and Core), was due to the count exceeding the predicted dilution and it is possible that the *Salmonella* levels were much higher. The appearance of *Salmonella* (though absent in the spread litter a few days before pick-up) first on day 0 in samples from the Bottom location (both sides A and B) and then subsequently on day 2 at other locations (Top B, core and sub core) indicates possible re-growth of *Salmonella* in the pile during these periods. However, by day 4 of the pile, *Salmonella* was not detected in any pile location (both MPN and presence/absence tests). The *Salmonella* detected in the subsequent spread litter was perhaps more a feature of the presence of chickens in this part of the shed. However due to the current observations of detection of *Salmonella* at different times and locations in the pile, carry-over of *Salmonella* cannot be ruled out.

During cycle 3 *Salmonella* was present before final pick-up in the litter. However, unlike the previous cycle, no quantifiable *Salmonella* was detected at any site of the pile on the first sampling date. The core sample on this date did yield *Salmonella* in the presence/absence test. *Salmonella* was present at quantifiable levels (> 110 MPN/g) in the Bottom sample (side A) collected on Day 3 of the pile cycle. There is again a possibility of re-growth occurring in Bottom A, the same location as cycle 2. While *Salmonella* was detected in the subsequently spread litter, the presence of chickens suggested that the *Salmonella* could have been sourced from the chickens.

The Bottom location side B had low levels of *Salmonella* at one time point in all three cycles – the only location to achieve this level of frequency. In total, there were 18 Bottom litter samples studied across all three cycles – with five of these 18 samples being positive for *Salmonella*. In contrast, of the matching 18 samples collected from the Top location, only one was positive for *Salmonella*.

In terms of serovars, *S.* Virchow was detected in the litter pile in cycles 1 and 2, with the litter prior to the cycle 1 pile up also containing *S.* Virchow. *S.* Zanzibar was detected in the litter pile in cycles 2 and 3. *S.* Sofia was isolated from the litter pile in cycle 3 and also the spread litter following litter pile-up cycle 3. As noted previously, chickens were present on this litter.

Campylobacter *die-off in piles through cycles 1, 2 and 3 - Laravale*

Figures 2.28 to 2.30 illustrate the levels of *Campylobacter* in three litter cycles at Laravale.

As with Donnybrook litter cycles *Campylobacter* was present in high levels, from a minimum of 10^3 to approximately 10^6 MPN/g in the litter prior to push-up in all three cycles. With one exception, *Campylobacter* was not detected in any pile position at any time in all three litter push-up cycles. The exception was that during cycle 3 *Campylobacter* was present at approximately 10^3 MPN/g one day after the pile was created in the Bottom Side B position. As with Donnybrook no carry-over of *Campylobacter* from the pile to the spread litter was detected. Even the presence of chickens in two cases (chicken day 13 after cycle 2 and chicken day 17 after cycle 3) did not result in any positive *Campylobacter* results.

Clostridium perfringens *die-off in piles through cycles 1, 2 and 3 – Laravale*

Figures 2.31 and 2.32 illustrate the levels of *Clostridium perfringens* for the latter two litter cycles at Laravale.

Cl. perfringens was present at around 10^4 CFU/g in all pile locations at all times in cycles 2 and 3. Interestingly, the spread litter from both push-up cycles 2 and 3 had higher levels of *Cl. perfringens* than was found in the various pile locations with counts of around 10^5 to 10^6 CFU/g. It should be noted that chickens were present on both of these occasions (days 13 and 17 for cycles 2 and 3 respectively).

Bacillus *die-off in piles through cycles 1, 2 and 3 – Laravale*

Figures 2.33 to 2.35 illustrate the levels of *Bacillus spp.* in three litter cycles at Laravale.

Bacillus species were absent in litter prior to push-up in cycle 1 (chicken day 49), with this cycle commencing after a full cleanout. However, *Bacillus* species were detected at all positions and all time points in the pile. The spread litter from this pile also contained *Bacillus* species.

Bacillus species were also present in all litter pile positions at both sampling days in cycle 2 and in the subsequently spread litter (Figure 2.34). Chickens of 13 days of age were present on this spread litter.

In cycle 3, *Bacillus* spp. were present in the spread litter prior to final chicken pick-up on day 48, in all pile positions at all sampling times and in the subsequently spread litter (Figure 2.35). Chickens of 17 days of age were present on this litter.

The only two species identified in the litter were *B. mycoides* and *B. cereus* (Table 2.3).

Figure 2.22 *E. coli* levels in litter at feeder and drinker (before and after litter piling) and in different positions in the litter pile over time (Laravale, cycle 1, November 2006). Chicken age when the litter was collected is shown in red font. There are three sampling days per pile position (with an absence of a bar indicating a level below the indicated detection level).

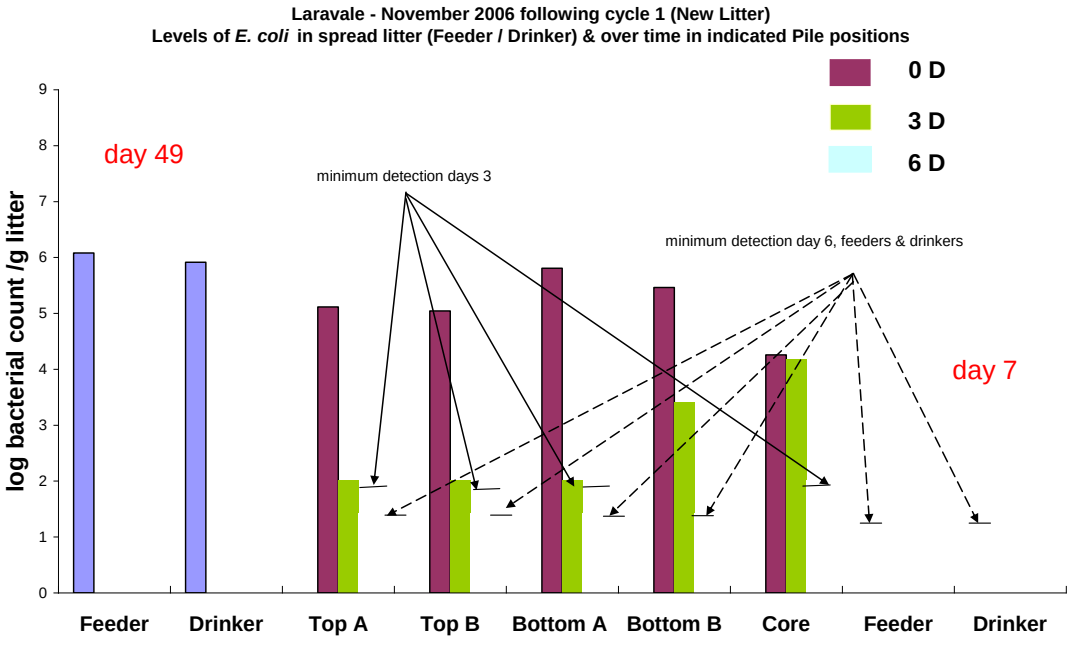


Figure 2.23 *E. coli* levels in litter at feeder and drinker (before and after litter piling) and in different positions in the litter pile over time (Laravale, cycle 2, January 2007). Chicken age when the litter was collected is shown in red font. There are three sampling days per pile position (with an absence of a bar indicating a level below the indicated detection level).

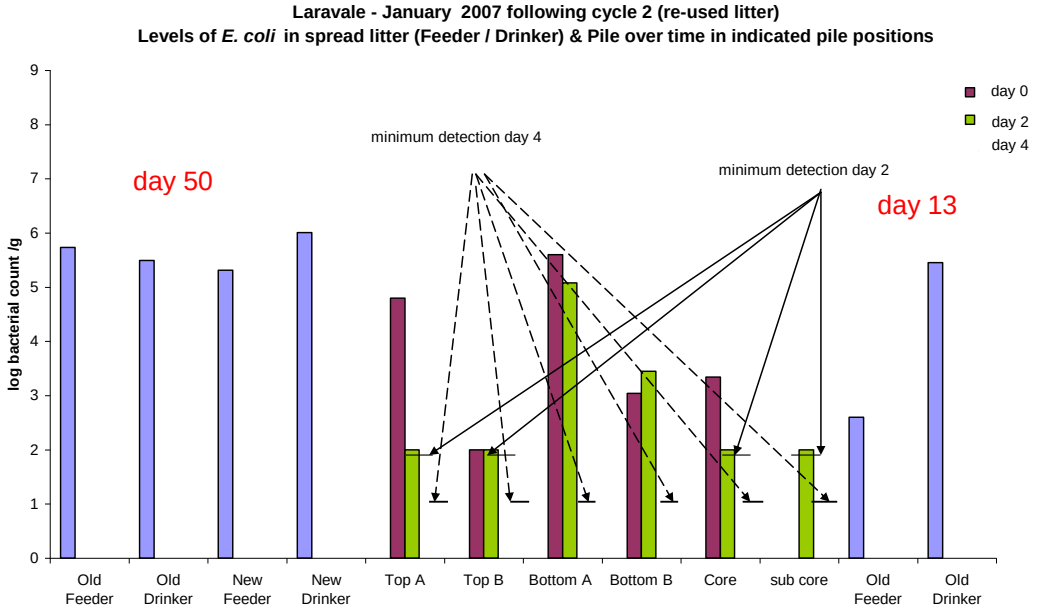


Figure 2.24 *E. coli* levels in litter at feeder and drinker (before and after litter piling) and in different positions in the litter pile over time (Laravale, cycle 3, April 2007). Chicken age when the litter was collected is shown in red font. There are three sampling days per pile position (with an absence of a bar indicating a level below the indicated detection level).

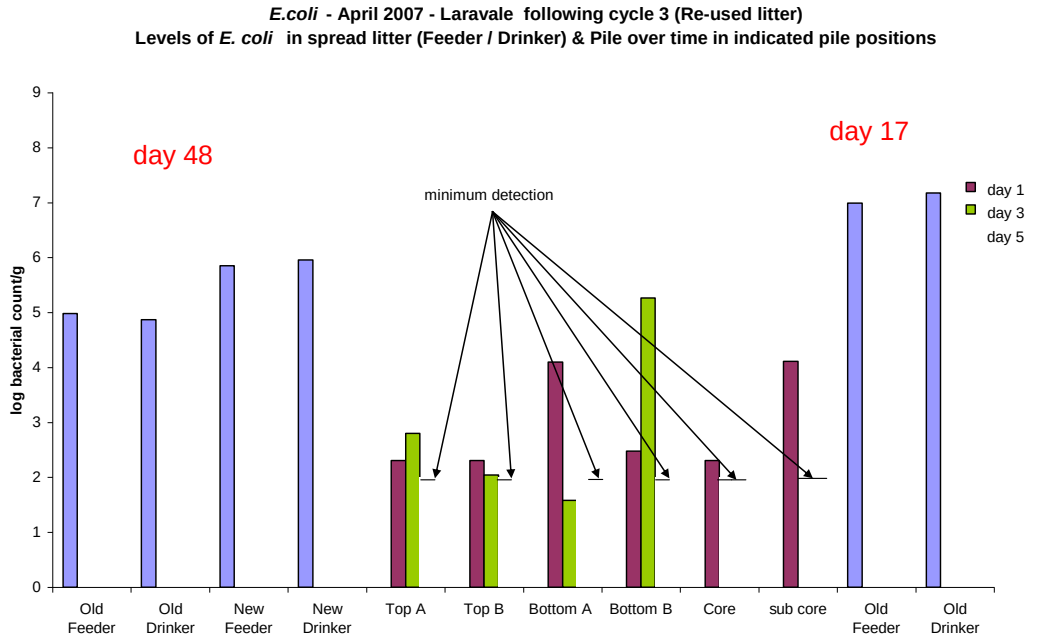


Figure 2.25 *Salmonella* levels in litter at feeder and drinker (before and after litter piling) and in different positions in the litter pile over time (Laravale, cycle 1, November 2006). Chicken age when the litter was collected is shown in red font. There are three sampling days per pile position (with an absence of a bar indicating a level below the indicated detection level). *Salmonella* serovars indicated in red font.

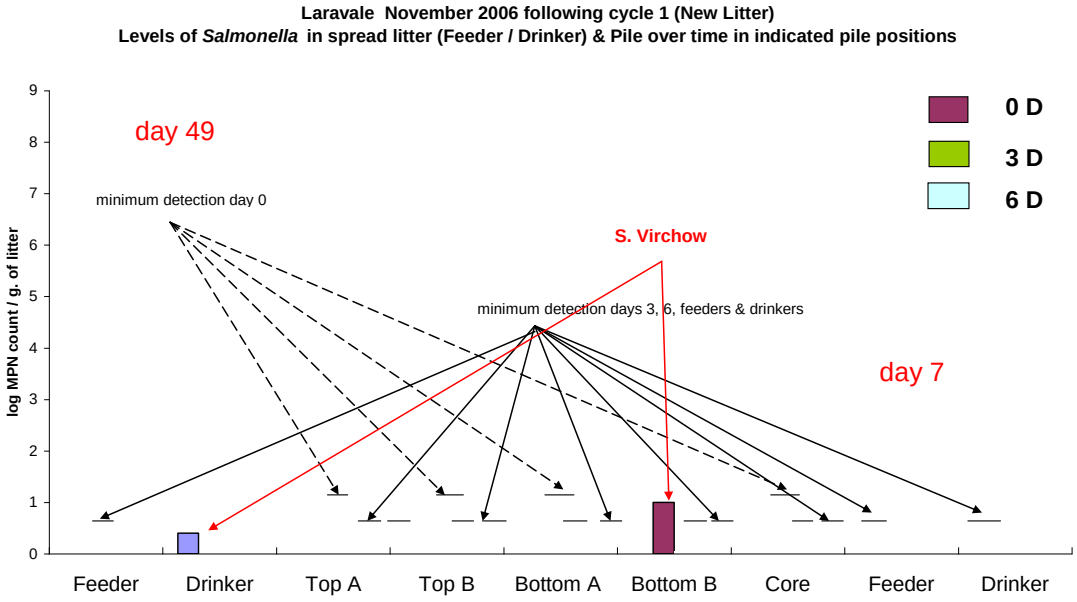
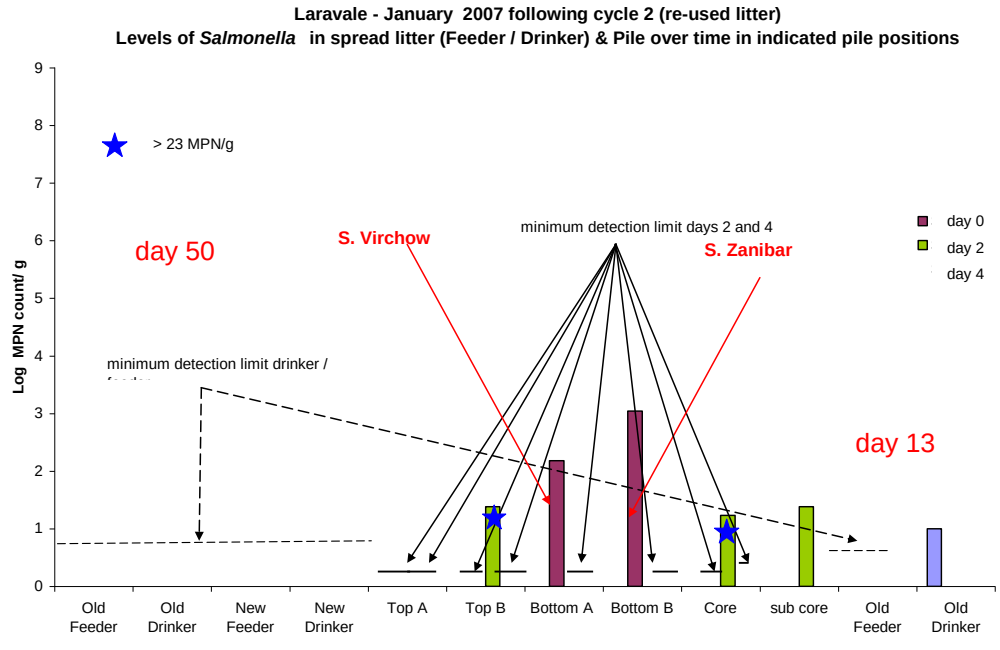
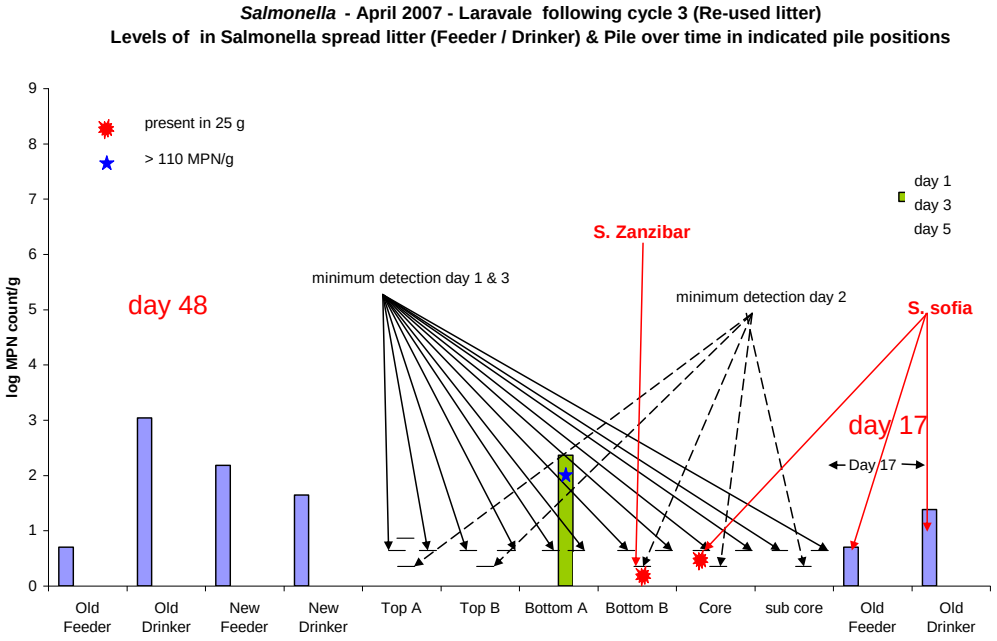


Figure 2.26 *Salmonella* levels in litter at feeder and drinker (before and after litter piling) and in different positions in the litter pile over time (Laravale, cycle 2, January 2007)**.** Chicken age when the litter was collected is shown in red font. There are three sampling days per pile position (with an absence of a bar indicating a level below the indicated detection level). The blue star indicates a count of >23 MPN/g as the end point was not reached. *Salmonella* serovars indicated in red font.



> 23 MPN/g due to a missed dilution thus higher count estimated

Figure 2.27 *Salmonella* levels in litter at feeder and drinker (before and after litter piling) and in different positions in the litter pile over time (Laravale, cycle 3, April 2007)**.** Chicken age when the litter was collected is shown in red font. There are three sampling days per pile position (with an absence of a bar indicating a level below the indicated detection level). The blue star indicates a count of >110 MPN/g as the end point was not reached. *Salmonella* serovars indicated in red font. The red star indicates a sample which was positive for *Salmonella* in the presence/absence test (25 g of litter) but where the levels were below the detection limit of the MPN method.



★ >110 MPN/g due to a missed dilution thus higher count estimated

Figure 2.28 *Campylobacter* levels in litter at feeder and drinker (before and after litter piling) and in different positions in the litter pile over time (Laravale, cycle 1, November 2006). Chicken age when the litter was collected is shown in red font. There are three sampling days per pile position (with an absence of a bar indicating a level below the indicated detection level).

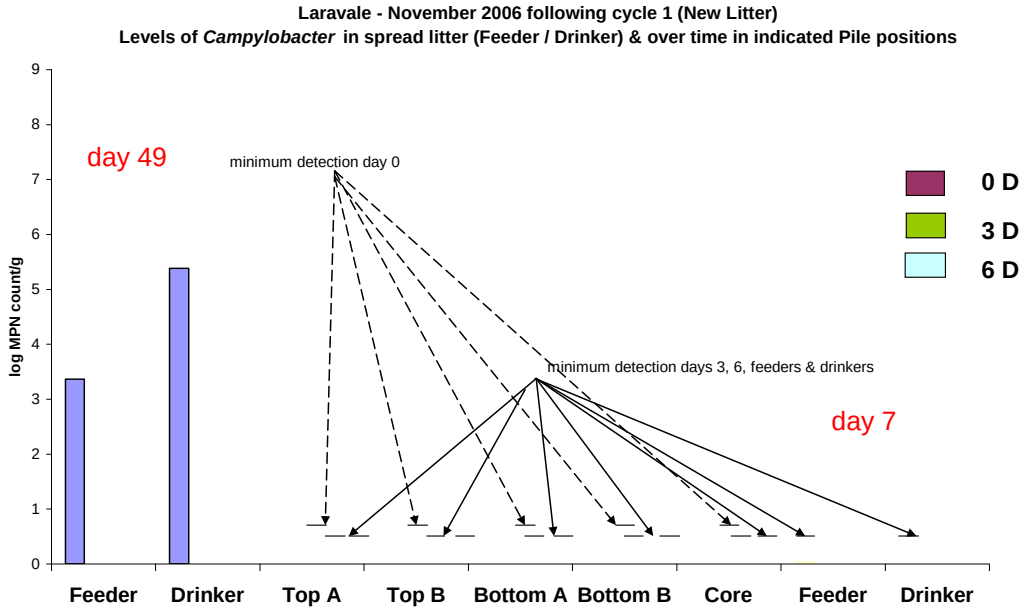


Figure 2.29 *Campylobacter* levels in litter at feeder and drinker (before and after litter piling) and in different positions in the litter pile over time (Laravale, cycle 2, January 2007)**.** Chicken age when the litter was collected is shown in red font. There are three sampling days per pile position (with an absence of a bar indicating a level below the indicated detection level).

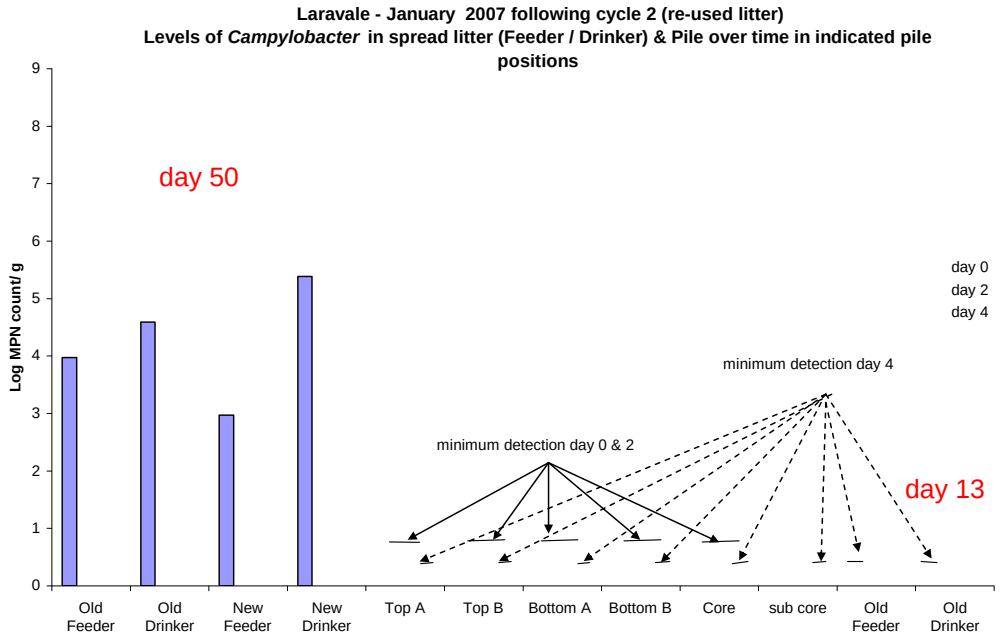


Figure 2.30 *Campylobacter* levels in litter at feeder and drinker (before and after litter piling) and in different positions in the litter pile over time (Laravale, cycle 3, April 2007)**.** Chicken age when the litter was collected is shown in red font. There are three sampling days per pile position (with an absence of a bar indicating a level below the indicated detection level).

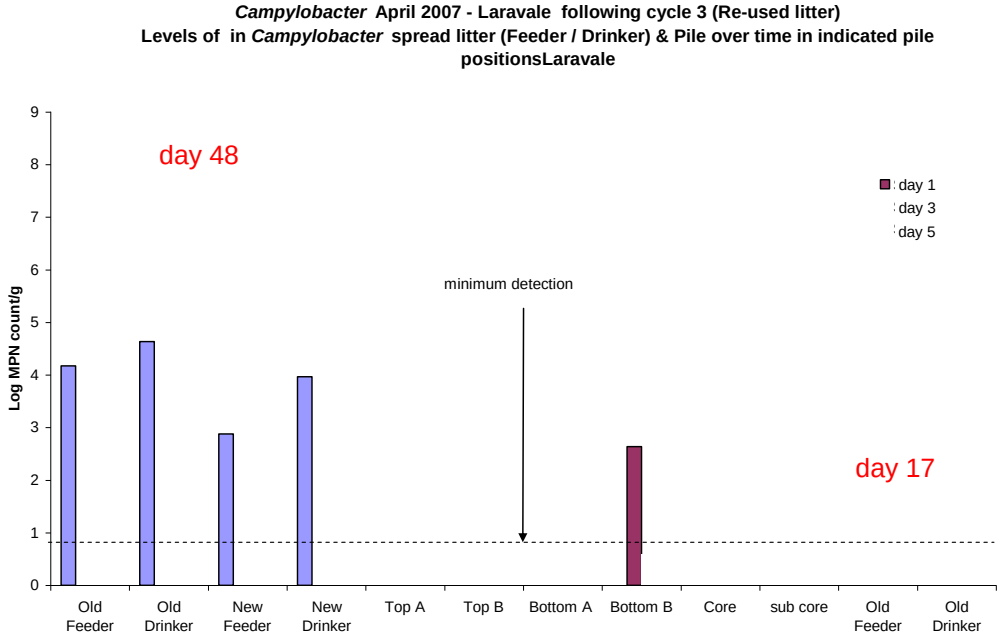


Figure 2.31 *Cl. perfringens* levels in litter at feeder and drinker (before and after litter piling) and in different positions in the litter pile over time (Laravale, cycle 2, January 2007). Chicken age when the litter was collected is shown in red font. There are two sampling days per pile position

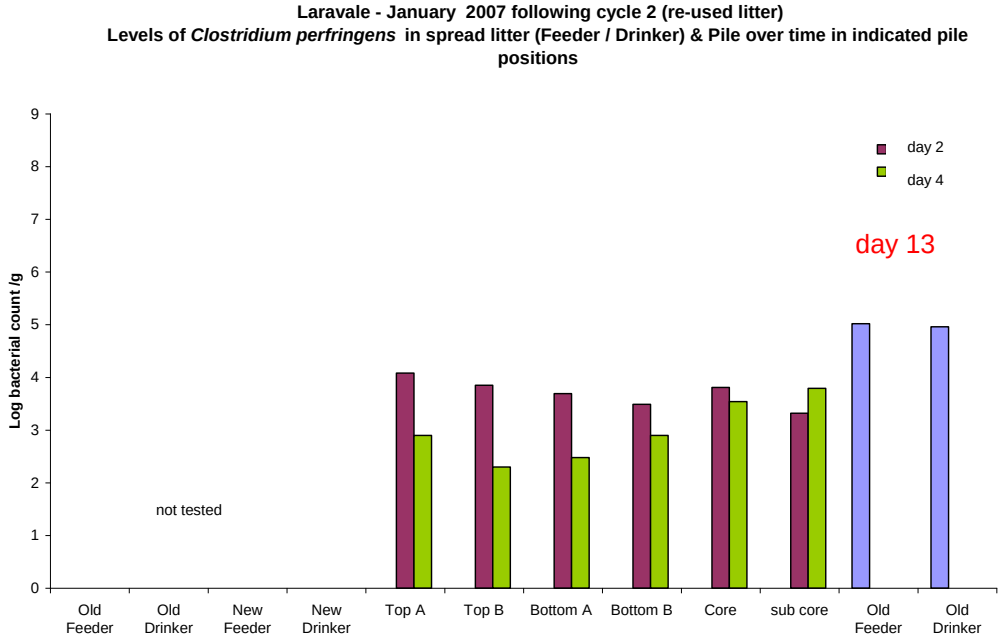


Figure 2.32 *Cl. perfringens* levels in litter at feeder and drinker (before and after litter piling) and in different positions in the litter pile over time (Laravale, cycle 3, April 2007). Chicken age when the litter was collected is shown in red font. There are three sampling days per pile position

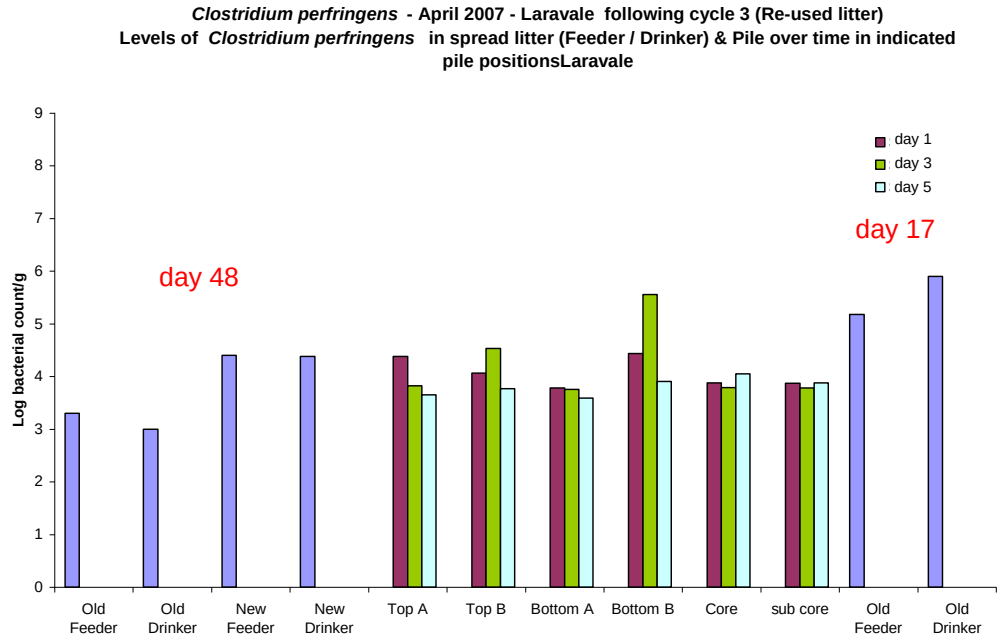


Figure 2.33 *Bacillus* levels in litter at feeder and drinker (before and after litter piling) and in different positions in the litter pile over time (Laravale, cycle 1, November 2006)**.** Chicken age when the litter was collected is shown in red font. There are three sampling days per pile position

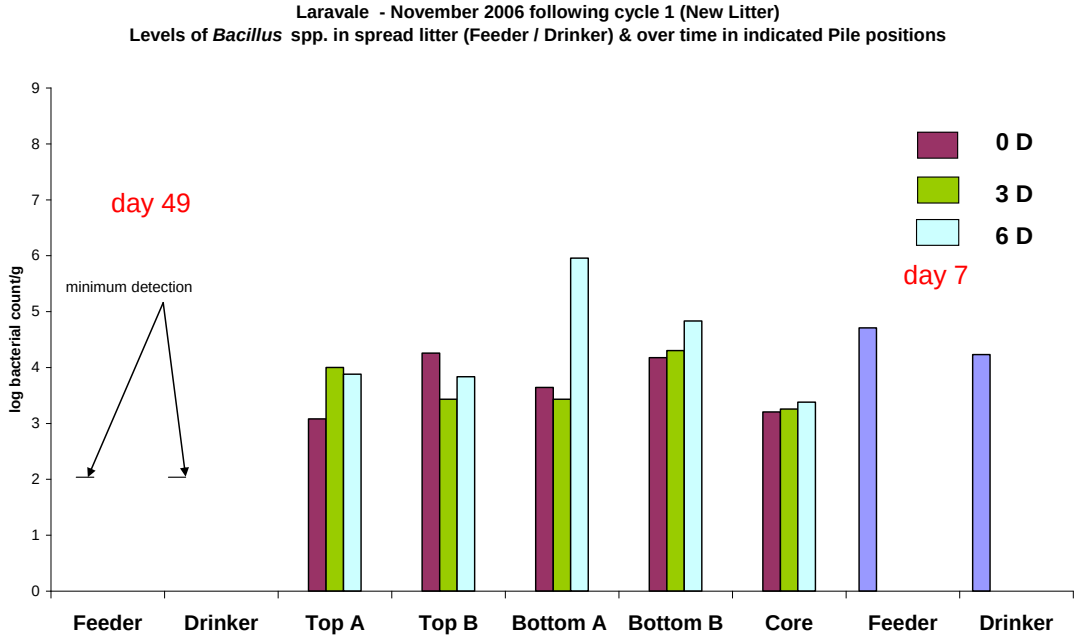


Figure 2.34 *Bacillus* levels in litter at feeder and drinker (before and after litter piling) and in different positions in the litter pile over time (Laravale, cycle 2 January 2007)**.** Chicken age when the litter was collected is shown in red font. There are two sampling days per pile position

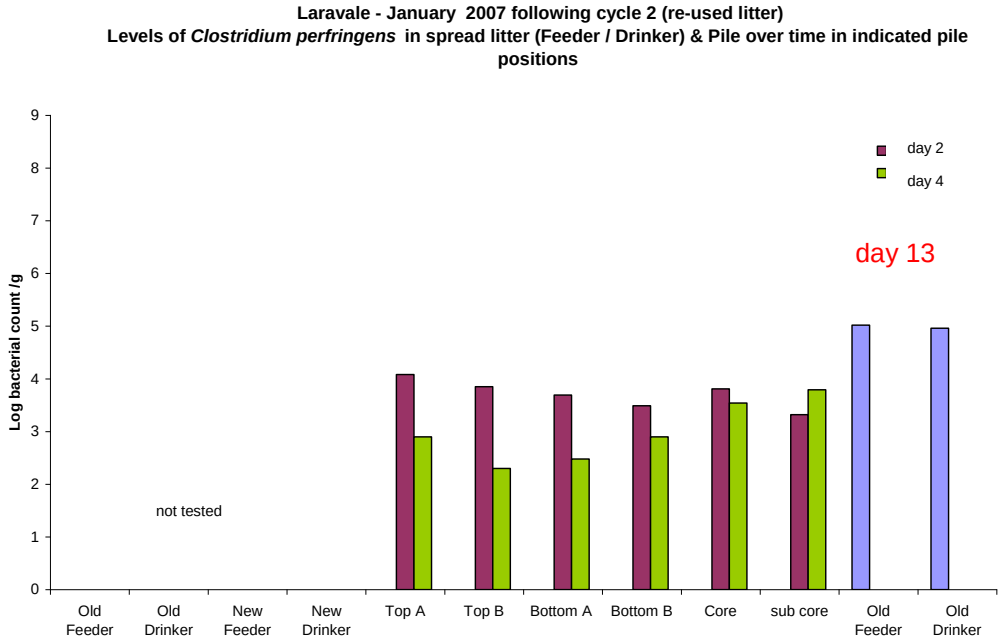


Figure 2.35 *Bacillus* levels in litter at feeder and drinker (before and after litter piling) and in different positions in the litter pile over time (Laravale, cycle 3, April 2007). Chicken age when the litter was collected is shown in red font. There are three sampling days per pile position

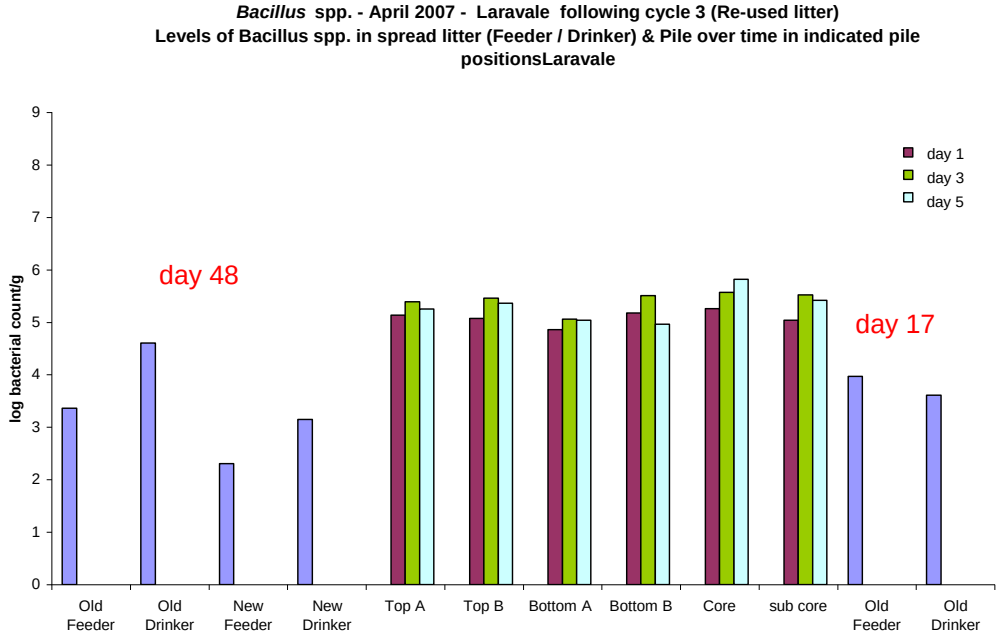


Table 2.3 Identification of *Bacillus spp* in litter from Laravale farm

Cycle – chicken / litter	Day of Pile	Position	Identification (Number of isolates)
litter cycle 1	1	Bottom Side B	*B. mycoides* (3)
	5	Core and Top Side B	*B. mycoides* (3)
chicken cycle 2	After piling	Spread litter	*B. cereus* (1) *B. mycoides* (2)
litter cycle 2	1	Top Side A	*B. cereus* (3) *B. mycoides* (1)
	5	Bottom Side A	*B. mycoides* (3)
chicken cycle 3	End of cycle – prior to piling	Spread litter	*B. cereus* (2) *B. mycoides* (1)
litter cycle 3	1 6	Bottom Side A Bottom Side A	*B. cereus* (2) *B. mycoides* (2)
chicken cycle 4	After piling	Spread litter	*B. cereus* (1) *B. mycoides* (1)

pH in piles through litter cycles 1, 2 and 3 – Laravale

Figures 2.36, 2.37 and 2.38 illustrate the pH levels before pick-up, through the litter pile and in the spread litter over three cycles. The pH levels recorded during all three piles were around 8.5 to 9.0, a level similar to that seen in the Donnybrook studies. As with the Donnybrook study, there was evidence of a reduction in pH levels over time at some sites – for example the Core site in cycle 2 (Figure 2.37).

It is worth noting that as soon as these piles were pushed up, there was an increase in the level of ammonia odour in the shed. It would seem that this ammonia was generated more from the aerobic surfaces of the pile. A gradual decrease in the ammonia odour over the life of the piles was noted.

aW in piles through litter cycles 1, 2 and 3 – Laravale

Figures 2.39, 2.40 and 2.41 illustrate aW levels before pick-up, through the litter pile and in the spread litter over three cycles. As with Donnybrook study, the water activity levels within the piles were around 1.00 in all pile locations for all three litter piles. The following were observed:

- The water activity of the spread litter after litter push-up cycles 1 and 2 (tested on day 7 and 13 of the next broiler flock respectively) was in the range of 0.75 – 0.8 (a zone where *Salmonella* growth is reported to be limited).
- This trend of lower water activity is seen in spread litter just prior to the third litter push up cycle (tested day 48 – broiler cycle). The litter under the feeder lines had water activity levels 0.7 and 0.75 while the litter under the drinkers had levels of 0.8 and 0.85.
- After the litter has been through the third litter push-up cycle, the spread litter (with chickens present) had water activity levels of 0.85 and 0.92 (with the lower value being in region regarded as a transition zone for *Salmonella* growth).

% moisture and relationship between aW and moisture in piles through cycles 1, 2 and 3 – Laravale

Figures 2.42, 2.43 and 2.44 illustrate both aW levels and moisture content before pick-up, through the litter pile and in the spread litter over three cycles.

The moisture content recorded for both the Bottom and Top locations in all three litter cycles for this farm were often high (35 – 40%). During litter cycle 1, we noted in our field observations that, on days 3 and 6 of the pile, the shed conditions were quite wet (see Figure 2.42 and Appendix 1). Whether the shed cleaning process, during proceeded while the litter pile-up process, contributed to this wetness is uncertain. During the pile-up process, the high moisture contents were also associated with high water activities (1.00 – 1.05). In general, it seems that the litter piles appear to be drying out over time. This is particularly noticeable with litter cycle 3 (Figure 2.44).

In contrast to the high moisture contents seen in the litter piles, the spread litter following all three litter piles were generally around the 20 – 25% level, with some drinker areas being wetter with 30% moisture. Again, this suggests a general drying of the litter over the litter pile-up process. Overall the piles demonstrated higher water activity and moisture levels than the spread litter.

During this study, we were able to log the shed relative humidity levels for litter cycle 3. In the previous cycles, as well as on the Donnybrook farm, we were limited to single observations while we were present in the shed. The continuous relative humidity records, along with the matching ambient temperature records, are shown in the lower half of Figure 2.44. This continuous monitoring showed that the relative humidity reached high levels (around 90%) during the evening hours for the first two days of the litter pile. There was an inverse relationship between relative humidity and temperature. The temperature peaks matched the lows of the relative humidity records. It is interesting to note that the monitoring showed that the day-time relative humidity levels recorded in cycle 3 were broadly similar to those recorded in cycles 1 and 2

There appears to be no relationship between the water activity and moisture when comparing the results of the Donnybrook and Laravale studies. In Donnybrook studies, a water activity of around 1.00 was associated with moisture contents of 20 – 25% in the cycle 2 litter pile (older litter) and approximately 30% in the cycle 1 litter pile. However, in the Laravale study, a water activity of greater than 1 was commonly seen through all pile treatments and was typically associated with a generally high moisture content of above 30%. There was an exception in that some pile locations in the last day of litter cycle 3 had water activity levels of around 1 but moisture contents of 20 to 25% (Top positions, day 5, Figure 2.44). These observations suggest that the piles at Laravale were wetter than Donnybrook and this may have to do with the cleaning (wetting) and piling processes occurring simultaneously at the Laravale farm.

As noted above, there appears to be a drying of the litter, in terms of % moisture, over the pile up process in some instances. This suggests that, though the "free water" available to microbes seem to persist at high levels (water activity levels of 1.00), the bound water component reduces with time during the pile-up process. This factor being more evident at Laravale, cycle 3 and may be a feature of aging litter.

However as soon as the litter is spread (see cycles 1 and 2, Figures 2.42 and 2.43) both water activity and moisture drastically reduce to levels that are generally regarded as not supporting microbial growth (water activity levels of 0.75 to 0.83 and % moisture levels of around 15-25%).

Figure 2.36 pH levels in litter at feeder and drinker (before and after litter piling) and in different positions in the litter pile over time (Laravale, cycle 1, November 2006). Broiler age when the litter was collected is shown in red font. There are three sampling days per pile position.

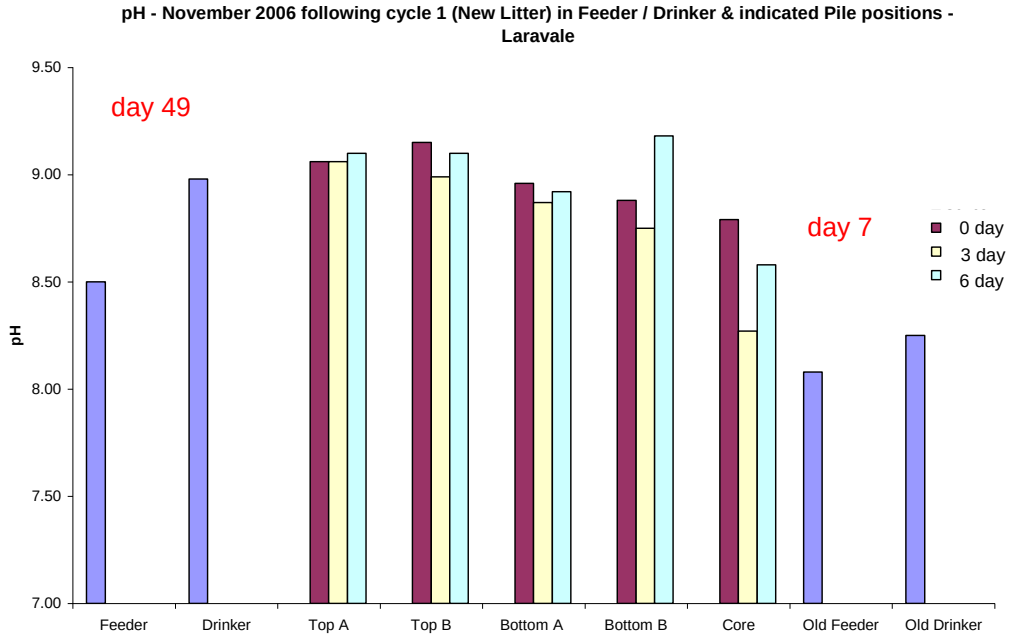


Figure 2.37 pH levels in litter at feeder and drinker (before and after litter piling) and in different positions in the litter pile over time (Laravale, cycle 2 January 2007). Broiler age when the litter was collected is shown in red font. There are three sampling days per pile position.

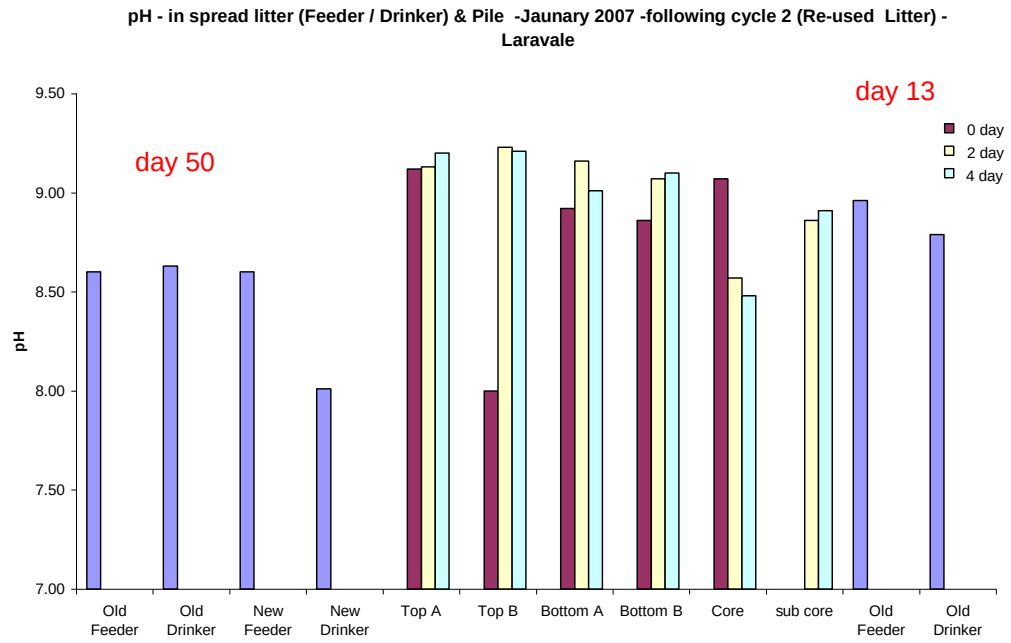


Figure 2.38 pH levels in litter at feeder and drinker (before and after litter piling) and in different positions in the litter pile over time (Laravale, cycle 3, April 2007). Broiler age when the litter was collected is shown in red font. There are three sampling days per pile position.

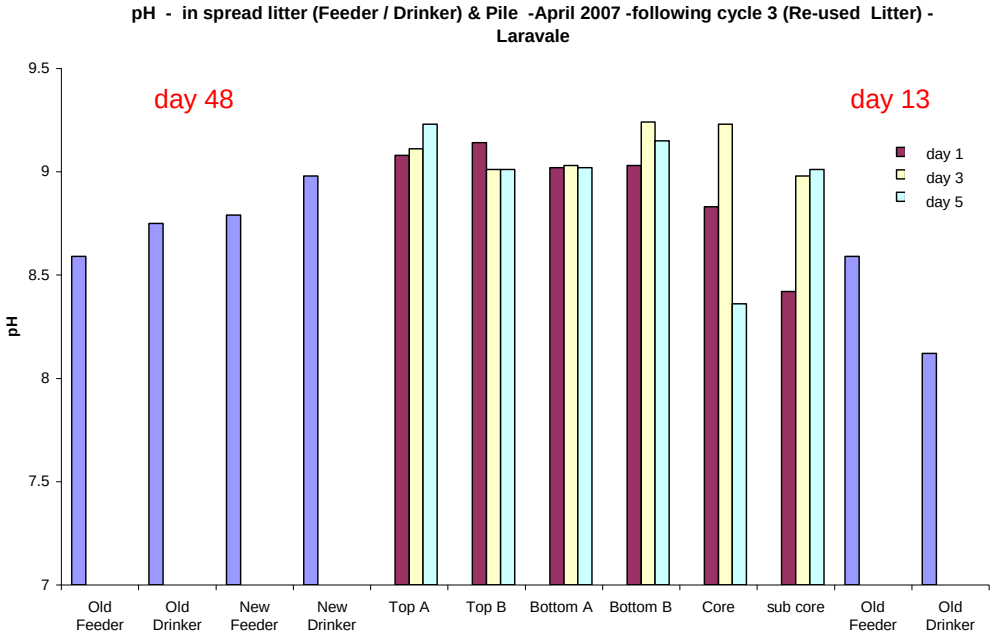


Figure 2.39 Water activity levels in litter at feeder and drinker (before and after litter piling) and in different positions in the litter pile over time (Laravale, cycle 1 November 2006).

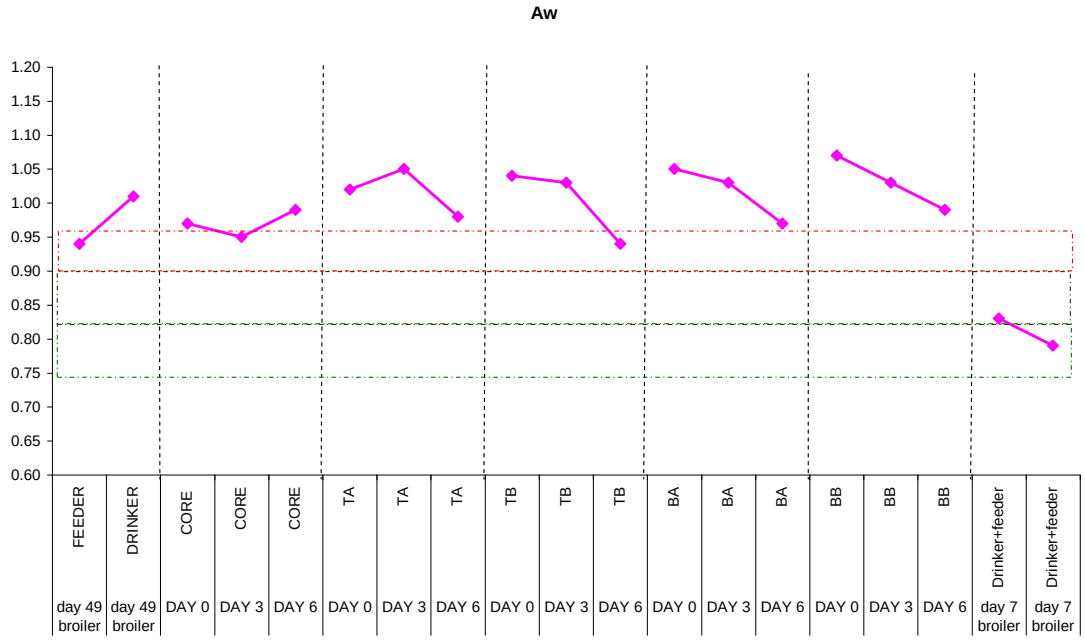


Figure 2.40 Water activity levels in litter at feeder and drinker (before and after litter piling) and in different positions in the litter pile over time (Laravale, cycle 2 January 2007)**.**

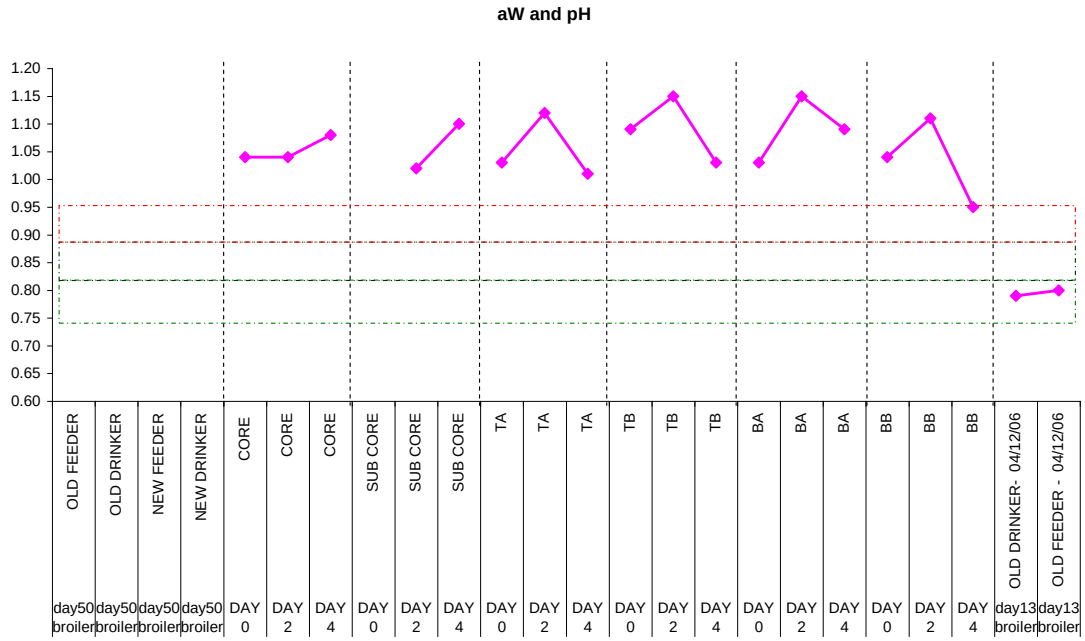


Figure 2.41 Water activity levels in litter at feeder and drinker (before and after litter piling) and in different positions in the litter pile and shed temperatures and % Relative Humidity over time (Laravale, cycle 3 April 2007)**.**

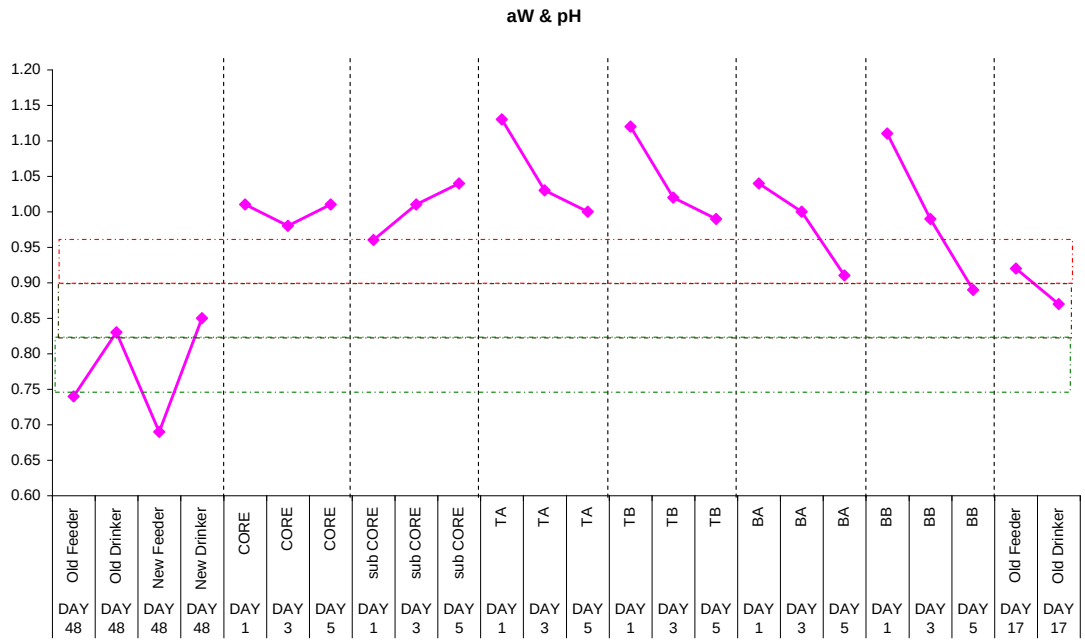


Figure 2.42 % Moisture and water activity levels in litter at feeder and drinker (before and after litter piling) and in different positions in the litter pile over time (Laravale, cycle 1 November 2006). The relative humidity in the shed is shown in blue font.

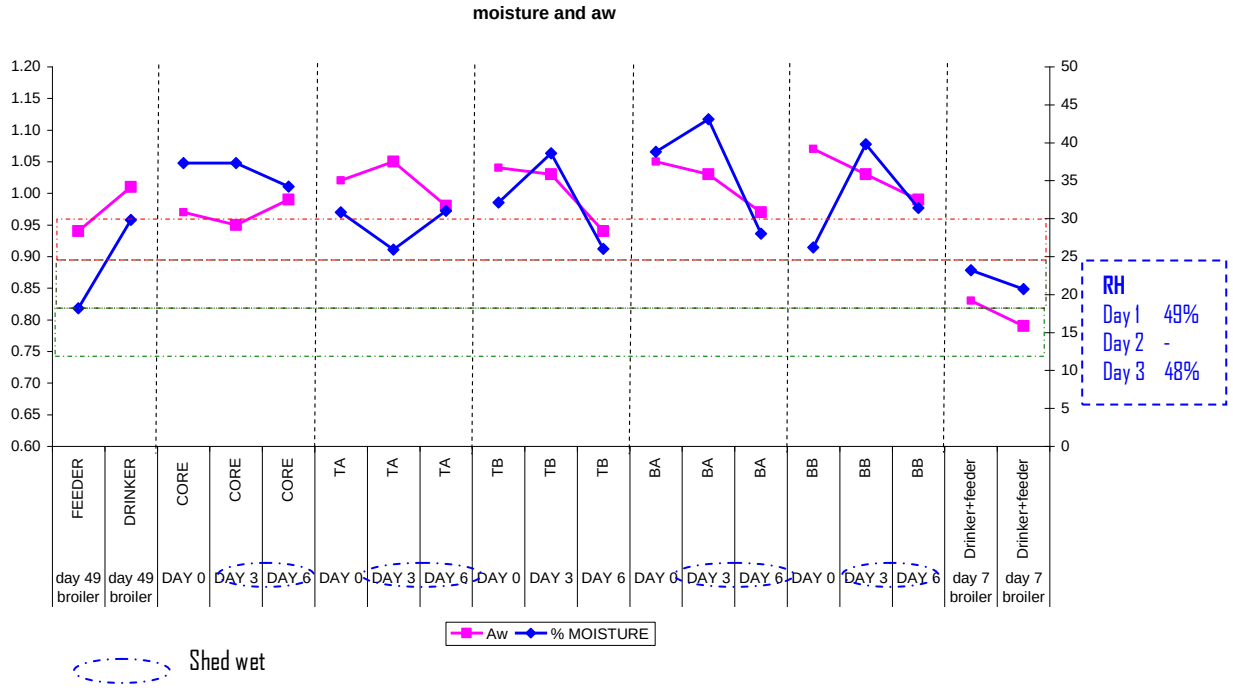


Figure 2.43 % Moisture levels and water activity levels in litter at feeder and drinker (before and after litter piling) and in different positions in the litter pile over time (Laravale, cycle 2 January 2007). The relative humidity in the shed is shown in blue font.

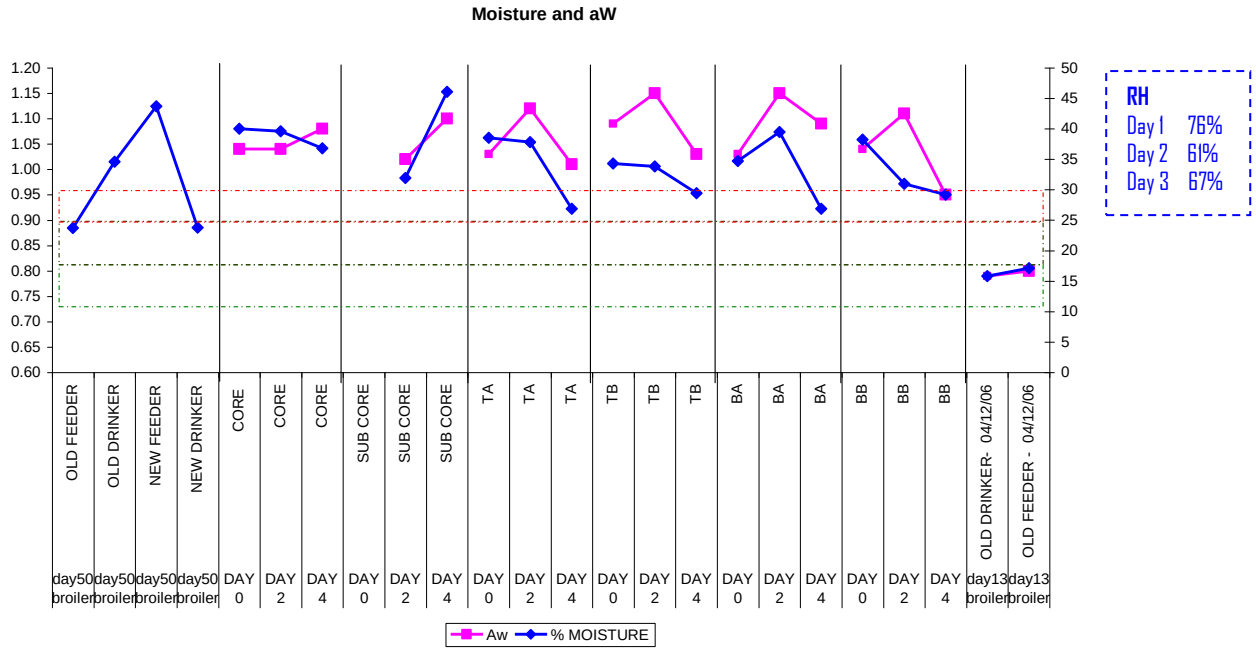
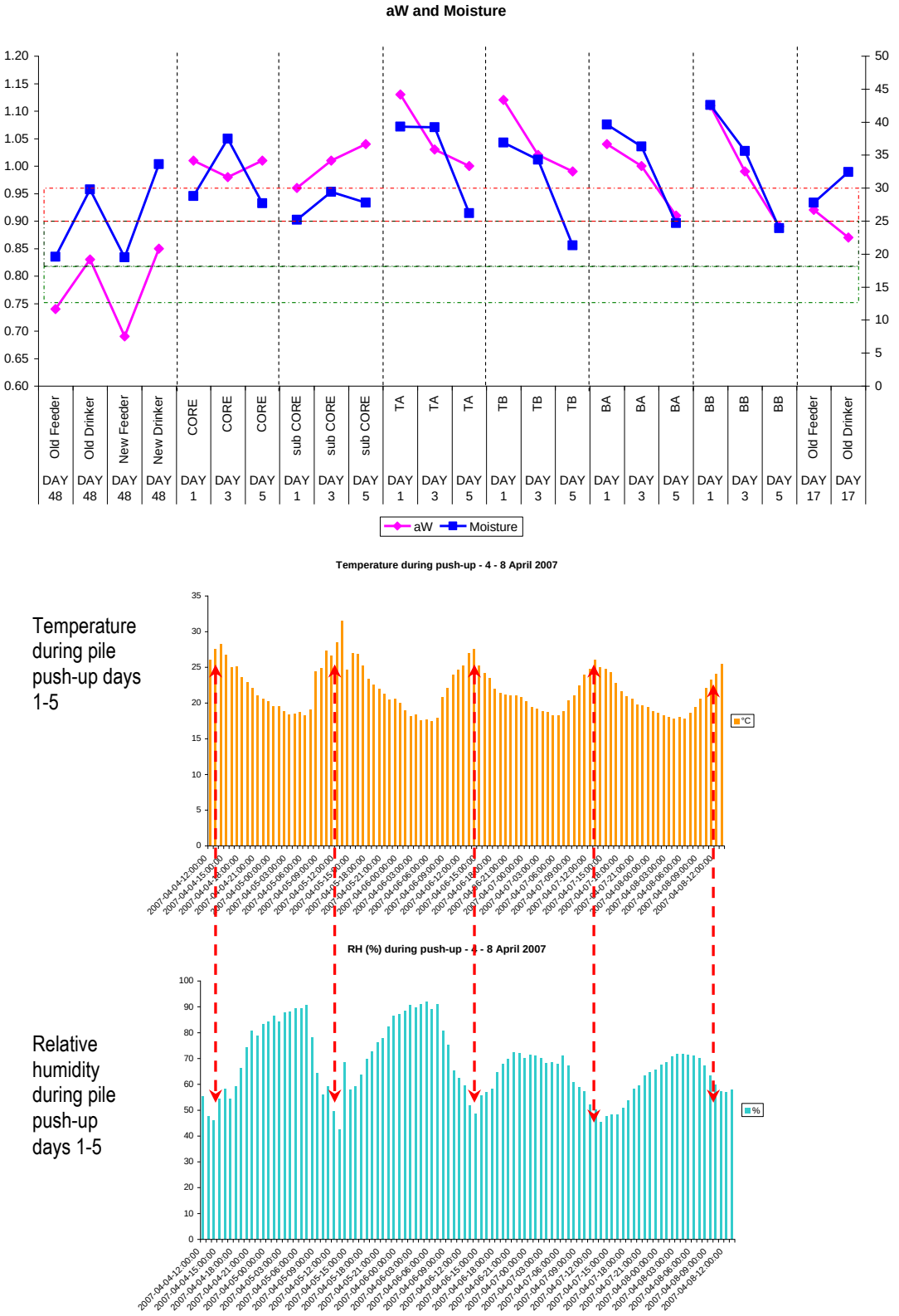


Figure 2.44 % Moisture levels in litter at feeder and drinker (before and after litter piling) and in different positions in the litter pile over time (Laravale, cycle 3 April 2007)**.** The bottom graphs show the results of continuous ambient temperature and relative humidity monitoring. The red arrows highlight the temperature peaks that are associated with the humidity lows.



Relationship between temperatures at different pile locations in cycles 1, 2 and 3 – Laravale

Figures 2.45, 2.46 and 2.47 present the temperature profiles at different pile locations over the life of the relevant pile through the three sequential cycles at the Laravale farm.

Unlike in the Donnybrook study, the second litter cycle involved a pile that contained litter from two broiler cycles. Hence this pile was much larger pile (height of 2.1 m, length of 28 m and width of 6 m) than the other two piles examined at Laravale. The piles for cycles 1 and 3 were more typical of the pile dimensions in the Donnybrook study (see Table 2.1). Despite this second pile being almost the double the length of the other two piles, the overall temperature maximums achieved across the three trials for the various locations did not vary much.

Prior to litter cycle 1, the litter was not pushed up for four days. This was evident in the different pattern in heating of the pile. A rapid rise in temperature occurred from a minimum of 30ºC on day 0 for the Top, Middle (100, 200 mm depths) and the Core (Top 1 m and Bottom 2m) (Figure 2.45). In contrast, the initial heating temperatures for the Bottom surface probes commenced from a minimum of 47-55ºC. Thus, this delay in push-up seemed to result in the bottom of the pile having a "head start" in terms of heating compared to the other regions of the pile.

Top measurements

In all three litter push up cycles, the surface temperatures at the top of the pile were approximately 50 - 65ºC. The temperatures at 100 mm depth generally lower than at 200 mm depths. Thus the surfaces were always slightly cooler than the inner regions of the pile. The temperatures were fairly stable over the life of the pile with the exception of the 100 mm depth probe in cycle 3. This position showed a marked decline in temperature over time. The general stability of the Top temperatures may have been assisted by the fact the top of the piles were physically close to the roof of the shed which perhaps acted as insulation. The decline seen in cycle 3 for the surface probe (100 mm) may have been a cooling effect due to wind effects from the openings in the shed side wall.

Middle measurements

Cycle 1 showed a different pattern between the two sides (A and B) (Figure 2.45). The probes on Side A (100 mm, 200 mm and 450 mm depths) all showed that the temperatures at these locations commenced at a relatively low 35ºC. Within a few hours, temperatures of 55 - 60ºC were reached. In contrast, the Side B probes (100 mm and 200 mm depths) showed that the initial temperatures at around 55 - 60ºC. The wires connecting into the data-logger dislodged after these initial readings and were re-connected the next day. This is why there is a gap in the data-line for these two probes (Figure 2.45). This side difference in the initial temperatures occurred in the pile that was pushed up 4 days after pick-up. Over time all the probes reached a peak temperature of around 60 - 65ºC. The 100 mm deep probes, on both sides A and B, showed a gradual drop in temperature to around 45 - 50ºC shortly after reaching the maximum temperature. In contrast, the two deeper probes, 200 mm (side B) and 450 mm (side A) remained stable at 60 - 65ºC. As previously noted, the shed was noticeably wet at times during this pile cycle – possibly influencing the temperature profiles of the pile. As well, wind movement patterns within the shed, as noted above, could also have an influence.

For cycles 2 and 3, the piles were created without delay. Even though the cycle 2 pile was considerably longer, the piles showed similar temperature profiles over time (Figures 2.46 and

2.47). The 100 mm and 200 mm surfaces probes reached temperatures around 60ºC and maintained these temperatures over the life of the pile.

Bottom measurements

During cycle 1, the Bottom surface probes showed temperatures that reached levels of 60 - 65ºC, a result that was also typical of the surface probes at the Top and Bottom positions (Figure 2.45). As with the other positions, the temperatures were better maintained at the 200 mm depths than at the 100 mm depths.

In contrast in cycle 2 (a longer pile), temperatures of 60 - 65ºC were better maintained at 100 mm (sides A and B) than at 200 mm (tested only side A). However, it is worth noting that the 200 mm deep probe showed an atypical pattern. It is possible that this probe was located in an air pocket rather than being in direct contact with the within the litter pile.

However during cycle 3 the depths of 100 and 200 mm did not seem to impact on the temperature profiles. All probes showed temperatures within the 40 - 60ºC range, although three probes showed a decline over time (100 mm and 200 mm Side A and 100 mm Side B) and one showed a rise over this range (200 mm Side B).

Core measurements

The heating pattern of the cores during cycles 2 and 3 were generally typical of those seen previously for pile cores - slow and steady increase in temperature and, in some instances, the cores did not reach temperatures as high as the some of the surface pile locations. Among all three cycles the highest temperature for a core observed was a steady 65ºC at the 1 m depth (Middle of pile) during cycle 3. This temperature of 65ºC was achieved also during cycle 1 at a depth of 1 m (Top of pile) but only during the late stages of the life of the pile.

In contrast, the lowest temperatures recorded for the core region was 45ºC at 2 m (Bottom of pile) in the large pile of cycle 2 as well as at 2 m (Bottom) in cycle 3 which was a typical pile in terms of dimensions. In fact, for cycle 2, all core probes - 1 m (Bottom side A), 1.5 m (Bottom side A and Middle side A) and 2 m (Bottom side A and Middle side A), all showed a similar pattern, a slow and steady increase from a minimum of 40ºC to a maximum of 45ºC over the life of the pile. Cycle 3 exhibited a similar pattern but the temperatures clustering into two categories with the Middle probes being somewhat higher than the Bottom probes (Figure 2.47).

Cycle 1 seem to be affected by the delay in push-up. Some locations began from a low temperature of around 35ºC (1 m Top side A and 2 m Bottom side A) while other locations began at around 50ºC (1 m and 2 m Middle side A). The 2 m core probes (Bottom side A and Middle side A) both only reached temperatures of around 45ºC. The other two core probes (1 m Top side A and 1 m Middle side A) reached higher temperatures – with the Top core getting to around 65ºC while the Middle core reached around 60ºC.

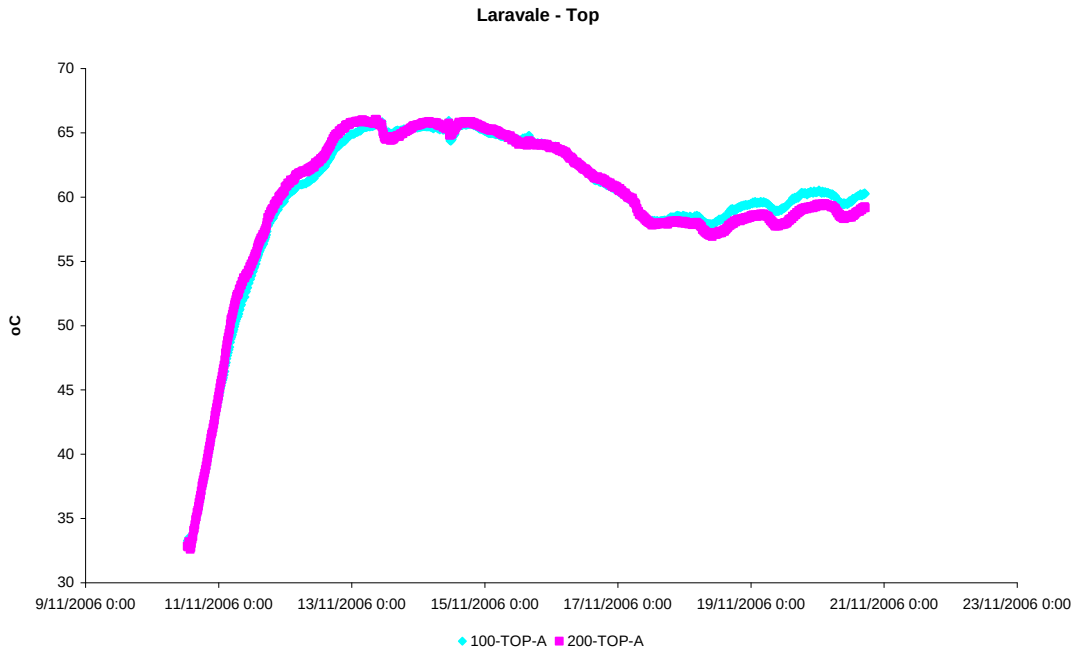
The slow heating trends of the core seen in these Laravale studies were also a feature of the Donnybrook piles.

Overall comments

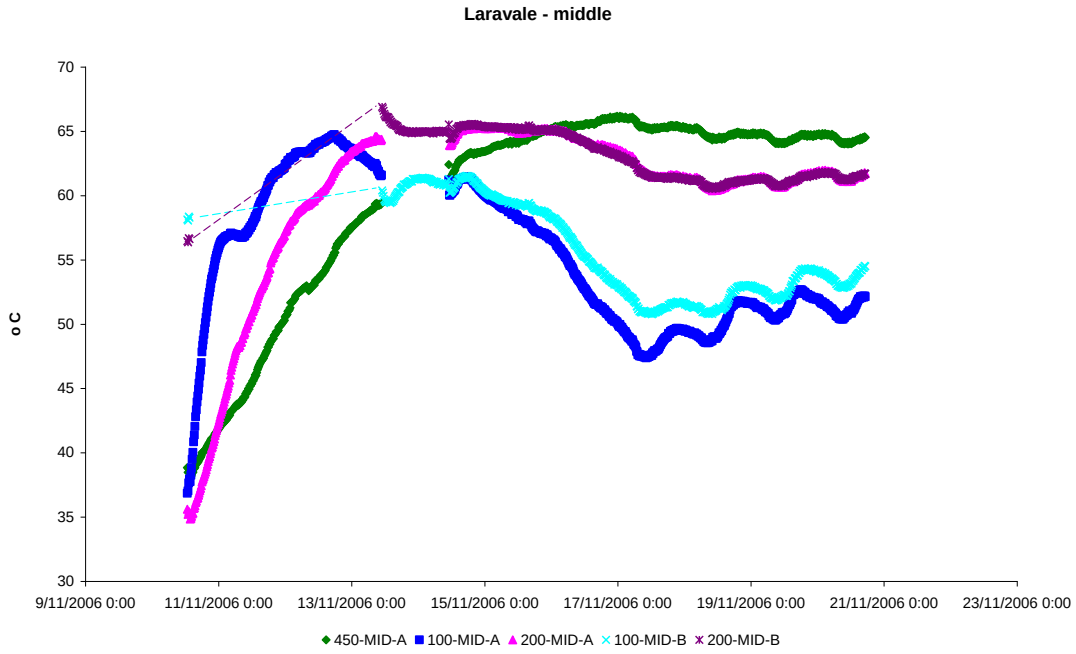
Overall the physical heating capacity of the piles seem to have been influenced by factors such as shed wetness, cooling breezes, the duration of push up from spread litter rather than actual dimensions of the piles or the locations of the farm (Laravale or Donnybrook).

Figure 2.45 Temperature profiles within litter pile over time (Laravale, cycle 1, November 2006). For each location, the depths at which the temperatures were recorded are shown. A) Near the top of the slope face of the pile; B) Middle of the slope face of the pile; C) Near the bottom of the slope face of the pile D) Core of the pile.

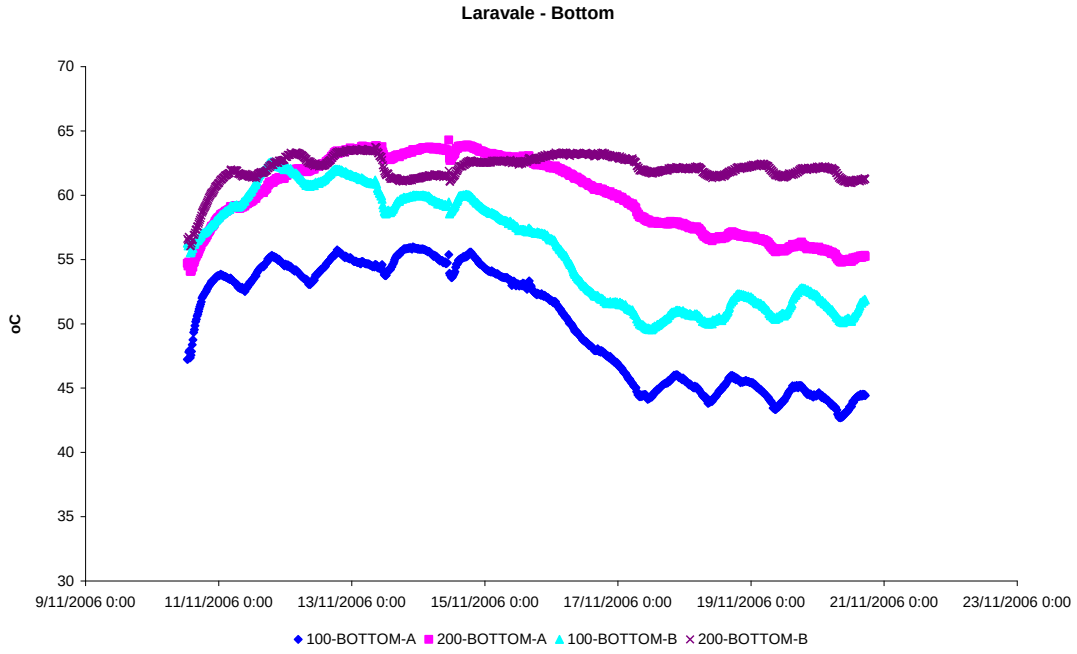
A.



B.



C.


Laravale - Bottom
oC
70
65
60
55
50
45
40
35
30
9/11/2006 0:00
11/11/2006 0:00
13/11/2006 0:00
15/11/2006 0:00
17/11/2006 0:00
19/11/2006 0:00
21/11/2006 0:00
23/11/2006 0:00
100-BOTTOM-A
200-BOTTOM-A
100-BOTTOM-B
200-BOTTOM-B

D.

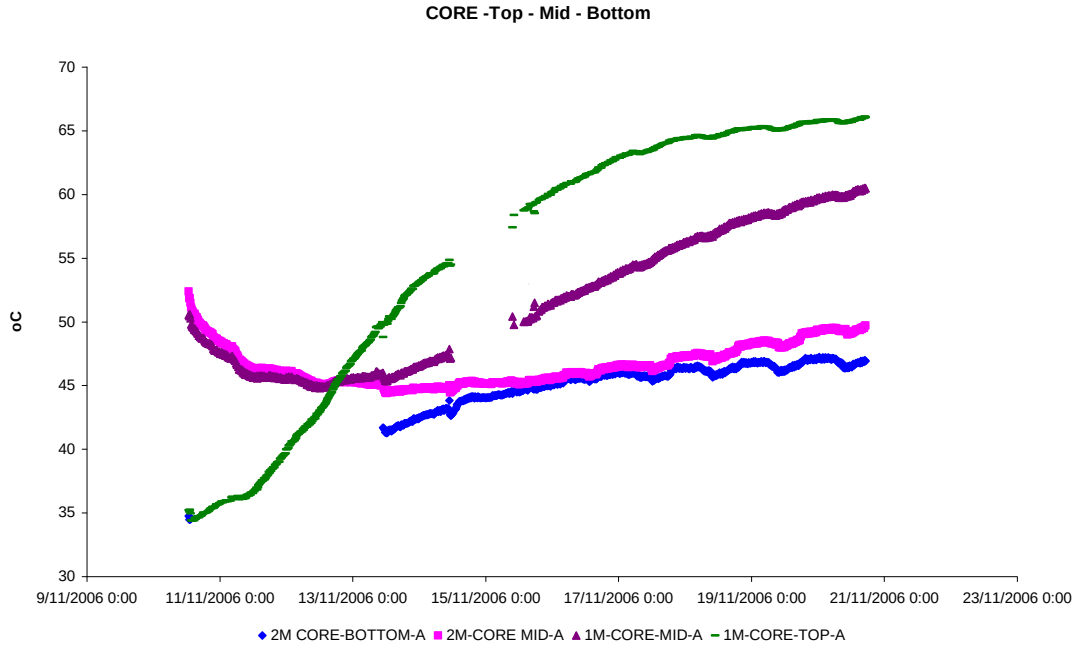
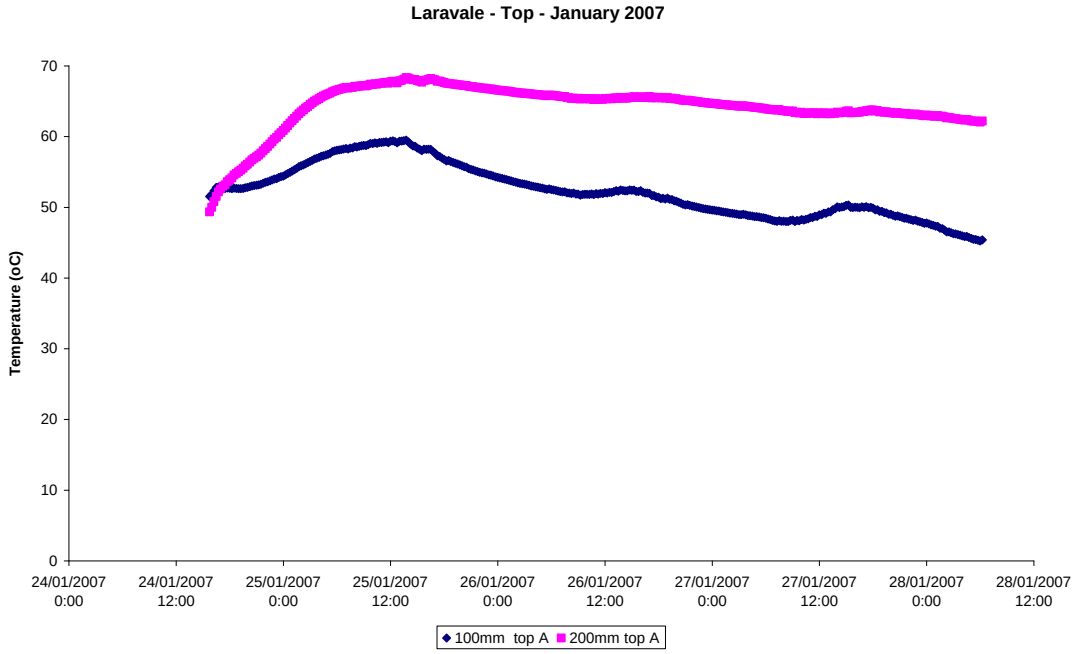
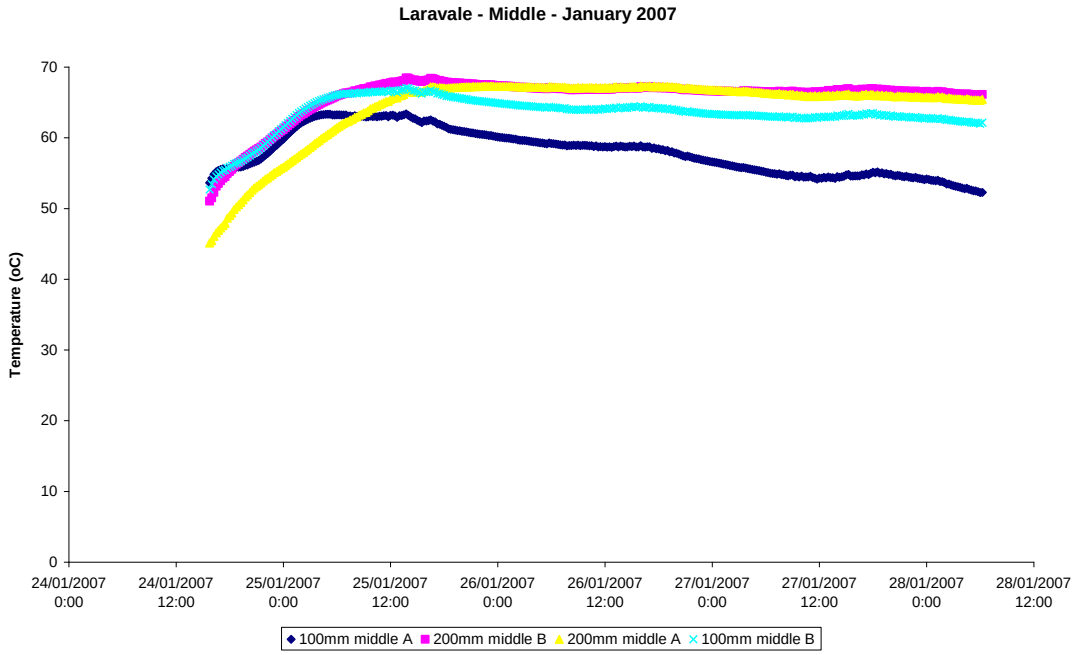

CORE -Top - Mid - Bottom
oC
70
65
60
55
50
45
40
35
30
9/11/2006 0:00
11/11/2006 0:00
13/11/2006 0:00
15/11/2006 0:00
17/11/2006 0:00
19/11/2006 0:00
21/11/2006 0:00
23/11/2006 0:00
2M CORE-BOTTOM-A
2M-CORE MID-A
1M-CORE-MID-A
1M-CORE-TOP-A

Figure 2.46 Temperature profiles within litter pile over time (Laravale, cycle 2, January 2007). For each location, the depths at which the temperatures were recorded are shown. A) Near the top of the slope face of the pile; B) Middle of the slope face of the pile; C) Near the bottom of the slope face of the pile D) Core of the pile.

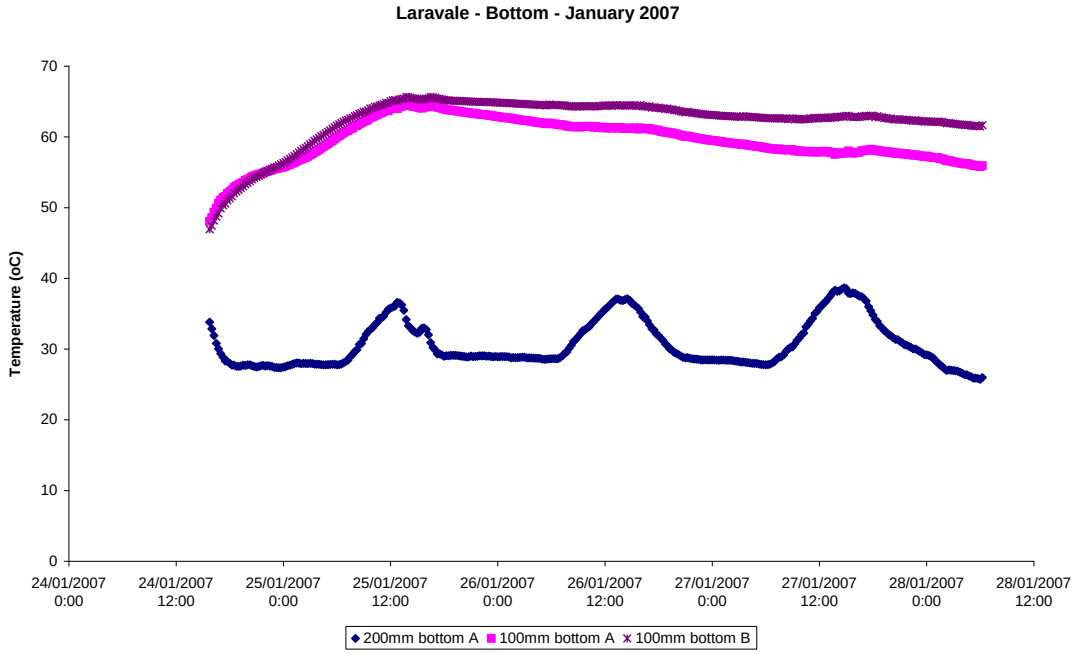
A.



B.



C.


Laravale - Bottom - January 2007
Temperature (oC)
70
60
50
40
30
20
10
0
24/01/2007 0:00
24/01/2007 12:00
25/01/2007 0:00
25/01/2007 12:00
26/01/2007 0:00
26/01/2007 12:00
27/01/2007 0:00
27/01/2007 12:00
28/01/2007 0:00
28/01/2007 12:00
200mm bottom A
100mm bottom A
100mm bottom B

D.

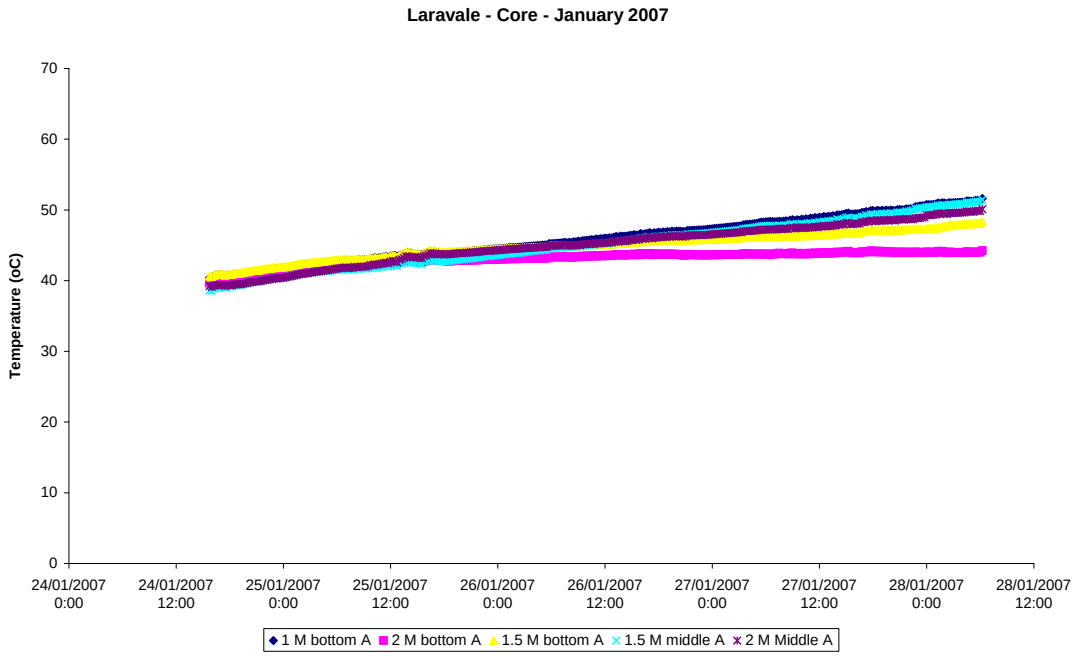
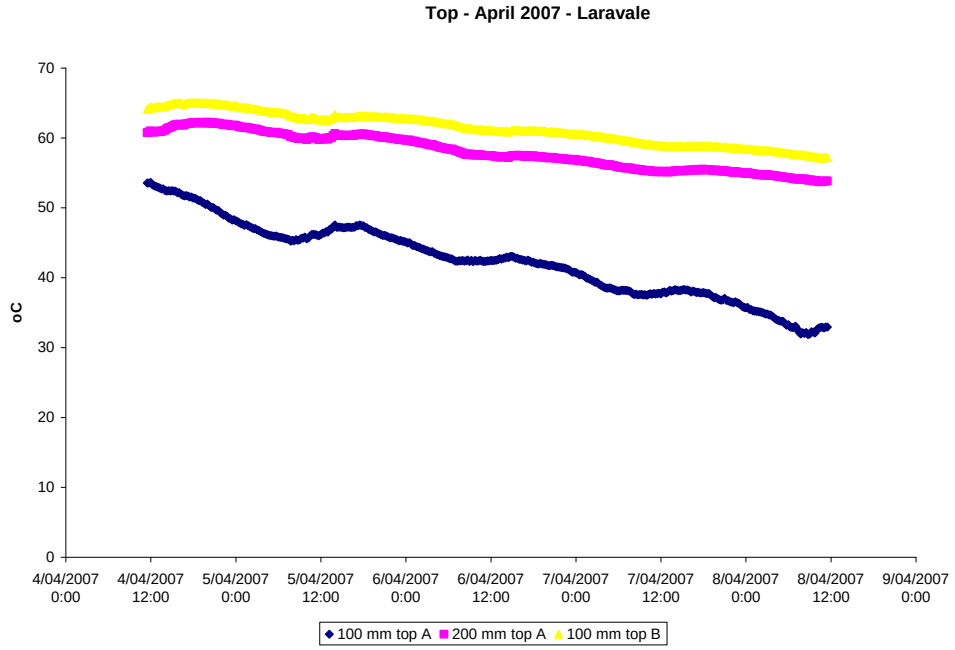
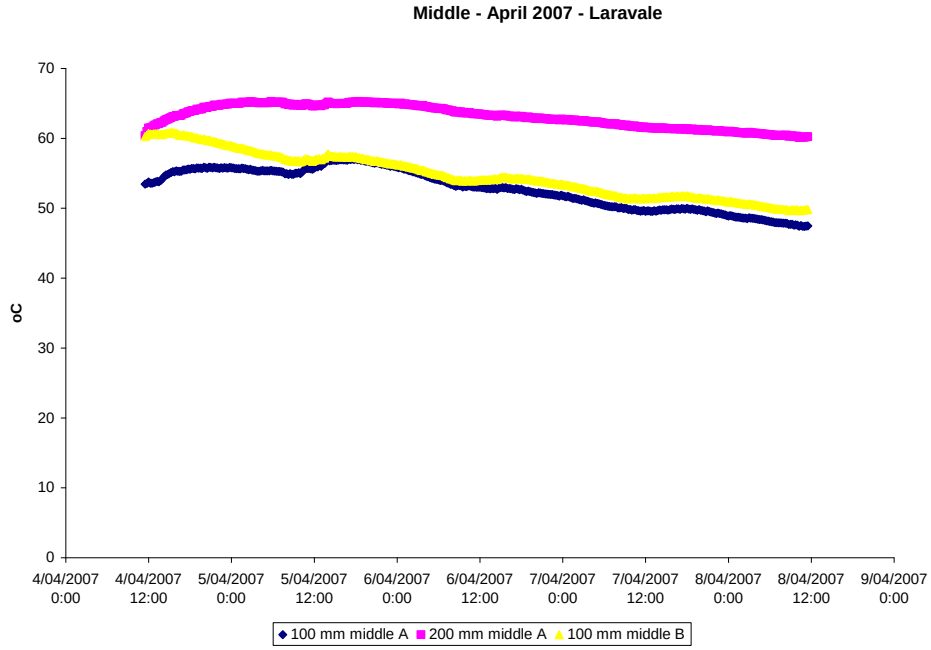

Laravale - Core - January 2007
Temperature (oC)
70
60
50
40
30
20
10
0
24/01/2007 0:00
24/01/2007 12:00
25/01/2007 0:00
25/01/2007 12:00
26/01/2007 0:00
26/01/2007 12:00
27/01/2007 0:00
27/01/2007 12:00
28/01/2007 0:00
28/01/2007 12:00
1 M bottom A
2 M bottom A
1.5 M bottom A
1.5 M middle A
2 M Middle A

Figure 2.47 Temperature profiles within litter pile over time (Laravale, cycle 3, April 2007). For each location, the depths at which the temperatures were recorded are shown. A) Near the top of the slope face of the pile; B) Middle of the slope face of the pile; C) Near the bottom of the slope face of the pile D) Core of the pile.

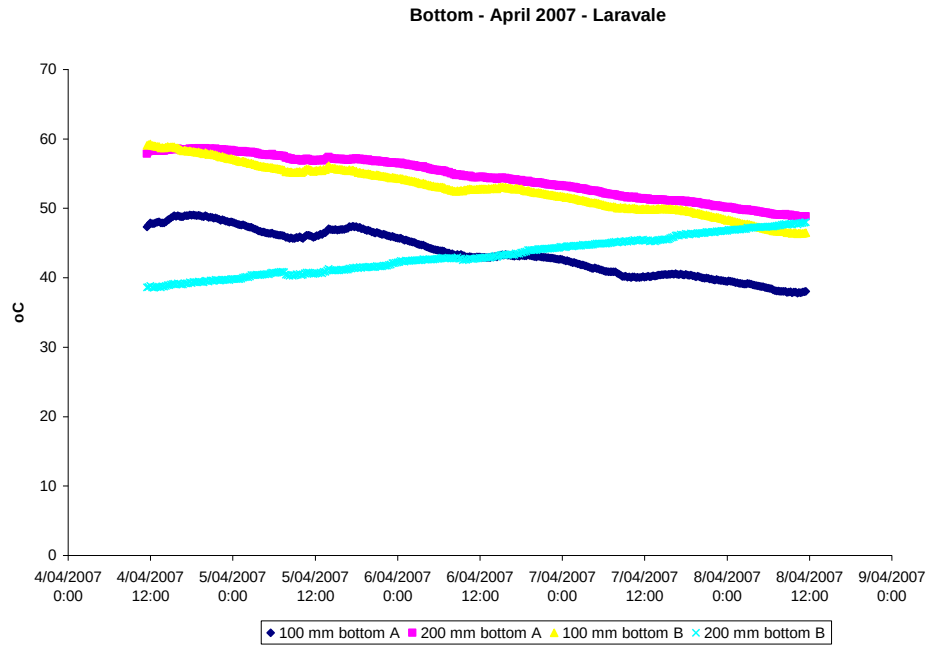
A.



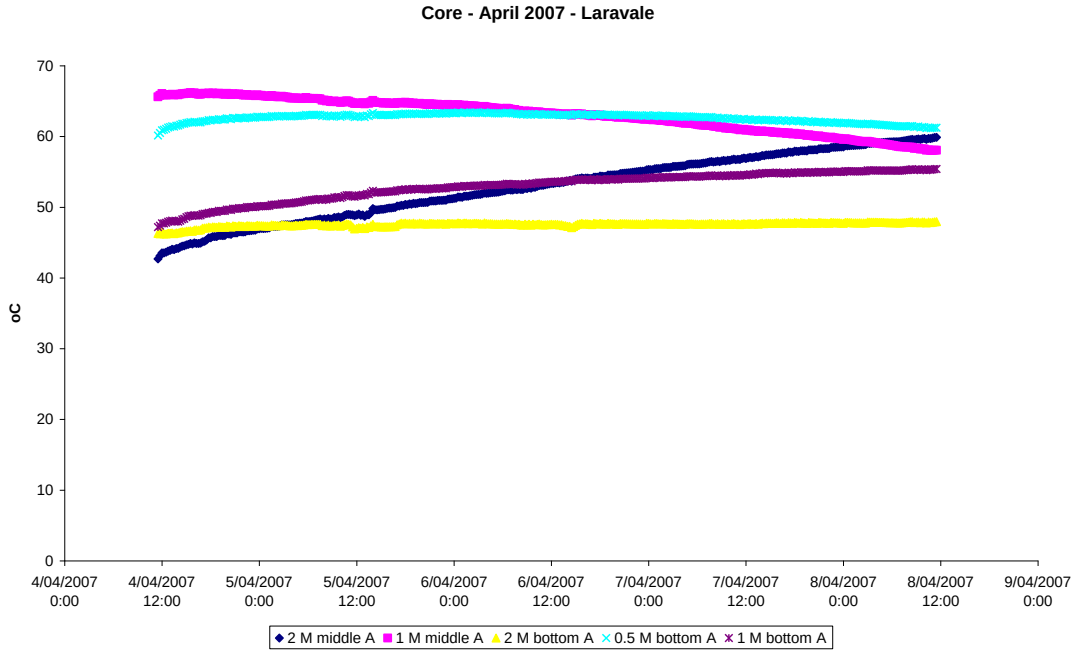
B.



C.


Bottom - April 2007 - Laravale
oC
70
60
50
40
30
20
10
0
4/04/2007 0:00
4/04/2007 12:00
5/04/2007 0:00
5/04/2007 12:00
6/04/2007 0:00
6/04/2007 12:00
7/04/2007 0:00
7/04/2007 12:00
8/04/2007 0:00
8/04/2007 12:00
9/04/2007 0:00
100 mm bottom A
200 mm bottom A
100 mm bottom B
200 mm bottom B

D.


Core - April 2007 - Laravale
oC
70
60
50
40
30
20
10
0
4/04/2007 0:00
4/04/2007 12:00
5/04/2007 0:00
5/04/2007 12:00
6/04/2007 0:00
6/04/2007 12:00
7/04/2007 0:00
7/04/2007 12:00
8/04/2007 0:00
8/04/2007 12:00
9/04/2007 0:00
2 M middle A
1 M middle A
2 M bottom A
0.5 M bottom A
1 M bottom A

Environmental studies on *Salmonella*

Tables 2.4 and 2.5 present the results of the environmental sampling for *Salmonella*. The sampling in the solid sided shed had to be stopped at Day 15 of the broiler cycle due to health problems in the flock. These health problems were not associated with the study but forced the study to be stopped.

No *Salmonella* was detected in the environment towards the end of the first study flock in the curtain sided shed. By Day 26 of the second flock, the litter (in both the brooder and grow-out ends) was positive for *Salmonella*. The litter then remained positive for all subsequent sampling sites. Despite the litter being positive, extensive testing of the rest of the shed did not detect *Salmonella*. All isolates from this shed were identified as S. Sofia.

In the solid-sided shed, the litter was positive for *Salmonella.* *Salmonella* was also detected in the dust on the drinker line, although only in the brooder end of the shed. In terms of serovars, S Sofia was present in the drinker and the re-used litter. S. Agona was present in the new litter. Only two samplings were possible in the following flock. In the first sampling (at Day 1 of age), all environmental samples did not yield *Salmonella*. By Day 15 of age, the litter (in both ends of the shed) was positive for *Salmonella* although no other environmental sample was positive. All isolates of *Salmonella* from the Day 15 sampling were *Salmonella* 4, 12, D; negative.

Table 2.4 Results of environmental sampling for *Salmonella* - Curtain Sided Shed. The Day columns show the age of the chickens flock in the shed. New indicates the litter at the brooder end of the shed which had fresh bedding. Old indicates the grow-out end of the shed – which had the re-used litter from the previous cycle.

Location Sampled	Day 39		Day 47		Spread-after push up		Day 12		Day 26		Day 33		Day 47		Day 54	
	New	Reuse	New	Reuse	New	Reuse	New	Reuse	New	Reuse	New	Reuse	New	Reuse	New	Reuse
Litter	-	-	-	-	-	-	-	N/A	+	+	+	+	+	+	+	+
Drinker 1	-	-	-	-	-	-	-	N/A	-	-	-	-	-	-	-	-
Drinker 2	-	-	-	-	-	-	-	N/A	-	-	-	-	-	-	-	-
Feeder 1	-	-	-	-	-	-	-	N/A	-	-	-	-	-	-	-	-
Feeder 2	-	-	-	-	-	-	-	N/A	-	-	-	-	-	-	-	-
Rope 1	-	-	-	-	-	-	-	N/A	-	-	-	-	-	-	-	-
Rope 2	-	-	-	-	-	-	-	N/A	-	-	-	-	-	-	-	-
Bottom Ledge 1	-	-	-	-	-	-	-	N/A	-	-	-	-	-	-	-	-
Bottom Ledge 2	-	-	-	-	-	-	-	N/A	-	-	-	-	-	-	-	-
Top Ledge 1	-	-	-	-	-	-	-	N/A	-	-	-	-	-	-	-	-
Top Ledge 2	-	-	-	-	-	-	-	N/A	-	-	-	-	-	-	-	-
Air Vent 1	-	-	-	-	-	-	-	N/A	-	-	-	-	-	-	-	-
Air Vent 2	-	-	-	-	-	-	-	N/A	-	-	-	-	-	-	-	-
Curtain	-	-	-	-	-	-	-	N/A	-	-	-	-	-	-	-	-
Feeder Pipe	N/A	-	N/A	-	N/A	-	N/A	N/A	N/A	-	N/A	-	N/A	-	N/A	-
Heater	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A	-	N/A

N/A = not applicable

Table 2.5 Results of environmental sampling for *Salmonella* - Solid Sided Shed. The Day columns show the age of the chickens flock in the shed. New indicates the litter at the brooder end of the shed which had fresh bedding. Old indicates the grow-out end of the shed – which had the re-used litter from the previous cycle.

	Day 47		Day 1		Day 15	
	New	Reuse	New	Reuse	New	Reuse
Litter	+	+	-	-	+	+
Drinker 1	+	-	-	-	-	-
Drinker 2	-	-	-	-	-	-
Feeder 1	-	-	-	-	-	-
Feeder 2	-	-	-	-	-	-
Rope 1	-	-	-	-	-	-
Rope 2	-	-	-	-	-	-
Bottom Ledge 1	-	-	-	-	-	-
Bottom Ledge 2	-	-	-	-	-	-
Top Ledge 1	-	-	-	-	-	-
Top Ledge 2	-	-	-	-	-	-
Air Vent 1	-	-	-	-	-	-
Air Vent 2	-	-	-	-	-	-
Curtain	-	-	-	-	-	-
Feeder Pipe	N/A	-	N/A	-	N/A	-
Heater	-	N/A	-	N/A	-	N/A

Spread litter studies

The results discussed in this section represent studies looking at the levels of key target bacteria as well as physical parameters in the two litter "types" present in the study sheds. As noted earlier, both farms examined in this study (Donnybrook and Laravale) employed a system where chickens were always placed on fresh bedding (in the brooder end of the shed). Depending upon the broiler cycle timing, the grow-out end of the shed consisted of re-used litter. This re-used litter had been subjected to the piling process described in the earlier section on Pile Studies. As noted in the Materials and Methods, the brooder end of the shed was termed N1 and N2 while the grow-out end of the shed was termed R1 and R2. A buffer zone, where no samples were collected, lay between the "new" and "re-use" ends.

For the Donnybrook farm, the grow-out end of the shed (R1 and R2) had litter from the cycle 2 litter push-up. For the Laravale farm, the grow-out end had litter from the cycle 6 litter push-up.

Appendix 1 describes field observations recorded on the different days of testing.

Levels of E. coli *at both Donnybrook and Laravale through a chicken production cycle*

Figure 2.48 shows the levels of *E. coli* though the cycle at Donnybrook. The levels of *E. coli* in the new and re-used litter prior to chicken placement (labeled "pre-chicks") were below the detection limit. By day 5 *E. coli* levels increased to 10^8 CFU/g of "new" litter following the introduction of the young chicks. In old litter there was no great increase in levels until the movement of chicks across into "re-used" litter end of the shed. From this time point onwards, there was little difference in the *E. coli* levels of the two litter types (i.e. N1 and N2 c.f. R1 and R2) from day 18 until day 52. On the last sampling (labeled "post chicks"), the final pick up had occurred a few hours prior to the sample collection. This absence of chickens had no apparent impact on the *E. coli* levels in the two litter types.

At Laravale (Figures 2.49), an overall similar pattern was seen. One difference was that the levels of *E. coli* in the "re-used" litter showed a rise from day 0 to day 7 and which continued until day 14 when the curtains were raised. However, after the movement of the chickens across in the grow-out end, the levels of *E. coli* in "re-used" litter reached around 10^6 CFU/g similar to the levels in the "new" litter before following a gradual downward trend.

Levels of Salmonella (and serovars present) at Donnybrook through a chicken production cycle

Figures 2.50 (for "new" litter) and 2.51 (for "reused" litter) show the change in levels of *Salmonella* (and serovars) across a chicken production cycle at the Donnybrook farm. *S.* Virchow and *S.* Chester were detected at low levels (below the detection limit of the MPN technique but detected in the presence/absence test) in the new litter before the placement of the chickens. Both of these serovars were detected on a number of occasions (at much higher levels) later in the cycle. No *Salmonella* were detected in the "re-use" litter prior to chick placement.

In week 1 and 2, high levels of *Salmonella* were found in the "new" litter. In contrast, *S.* Chester was detected in the "re-use" litter at only a low level (below the quantifiable levels of the MPN technique) at week 1 when chickens were present in the shed but not yet in the grow-out end of the shed. At week 2, the "re-use" litter samples were negative for *Salmonella*. The dominant serovars of *Salmonella* in the "new" litter for first two weeks of the cycle were *S.* Chester, *S.* Seneftenberg and *S.* Singapore. *S.* Chester was commonly isolated throughout the rest of the cycle from the "new" litter.

Once the curtains were raised (week 3), the *Salmonella* levels in the "re-used" litter rose to levels similar to that in the "new" litter. The serovars detected in the "new" litter were *S.* Chester and *S.* Singapore while *S.* Chester and *S.* Zanzibar were detected in the "re-used" litter. For weeks 4, 5 and 6, the *Salmonella* levels in both litter types showed the same trend – a fall at weeks 4 and 5 followed by a rise at week 6. A range of serovars was found in both types of litter – with *S.* Chester and *S.* Sofia being in both litter types. Over the last three sampling weeks, the level of *Salmonella* in the N1 "new" litter was consistently lower than that in the other three litters (N2 and the two "re-used" litter samples R1 and R2).

The "re-used" litter yielded predominantly *S.* Chester and *S.* Sofia over the period of the cycle. Only one other serovar (*S.* Zanibar) was detected and that was at a single time point. In the "new" litter, while the common serovars were again *S.* Chester and *S.* Sofia, additional serovars were detected on at least two occasions – *S.* Seneftenberg, *S.* Singapore and *S.* Virchow.

Levels of Salmonella *(and serovars present) at Laravale through a chicken production cycle*

Figures 2.52 (for "new" litter) and 2.53 (for "reused" litter) show the change in levels of *Salmonella* (and serovars) across a chicken production cycle at the Laravale farm. *S.* Agona was detected in "new litter" prior to chicken placement. Interestingly, *S.* Agona was only detected at one time point in the subsequent cycle. This contrast with the situation at the Donnybrook farm where the serovars detected prior to chicken placement in the "new" litter (*S.* Chester and *S.* Virchow) were repeatedly detected later in the cycle. The "re-used" litter did not yield *Salmonella* prior to chicken placement.

By week 2, both of the "new" litter samples had high counts of *Salmonella* (around 10^5 MPN/g) while one of the "re-use" litters was negative and the other had only a low level of *Salmonella* (around 10^2 MPN/g). *S.* Singapore and *S.* Sofia were the serovars detected in both types of litter.

For the rest of the cycle, the *Salmonella* levels in the "re-use" litter were consistently lower than those seen in the "new" litter. A fall and rise pattern in *Salmonella* levels occurred in the "new" litter (see Figure 2.52) while the levels in the "re-use" litter tended to be fairly low and fairly stable (see Figure 2.53). There was a general downwards trend in *Salmonella* levels in all litter types from week 7 to week 8. Indeed, the "re-use" litter levels were below the minimum detection limit (0.3 MPN per g). The R2 "re-use" litter did yield *S.* Montevideo (a serovar not previously seen in this cycle) in the presence/absence test.

Overall comments on Salmonella

The overall results from the *Salmonella* work suggests that on both farms there was a tendency for the "new" litter to support

- a greater variety of serovars
- a higher frequency of these serovars across the cycle
- a higher population

of *Salmonella* as compared with "re-use" litter.

Levels of Campylobacter at both Donnybrook and Laravale through the chicken production cycle

Figure 2.54 shows the levels of *Campylobacter* though the cycle at Donnybrook. *Campylobacter* was not detected till day 38 when one sample ("re-use" litter – R2) was positive at a relatively low level (240 MPN/g). By day 45, *Campylobacter* was detected in all samples, and appeared more or less uniformly distributed in litter across the shed. At both day 38 and day 45, pick-ups of chickens (around 15,000 and 3,000 chickens respectively) occurred and there would have been cross contamination across the zones of "new" and "re-use" areas as defined for this study.

By day 52 (just before final pick-up), *Campylobacter* levels were highest at the middle zones of the shed at ("re-use" litter R1 and "new" litter N2). Overall, the levels of *Campylobacter* in litter across days 45 to 52 were in the range of 10^4 to 10^5 MPN/g.

The litter was tested for the last time at around 10 hours after final chicken pick- up occurred. While one "new" litter sample (N1) still supported the high levels of *Campylobacter* (around 150,000 MPN/g), a drop off in levels was seen in the other litter samples (with counts of 430, 4,300 and 230 MPN/g in N2, R1 and R2 litters respectively).

At Laravale, a more or less similar pattern in *Campylobacter* levels was observed (See Figure 2.55). The first detection of *Campylobacter* (at day 42) was a week later than that seen at Donnybrook. At this first detection, the highest levels of Campylobacter were observed in the "re-use" litter.

There was a large pick-up on day 35 (16,500 birds), followed by another pick-up on day 42 (2,000 birds) and a final pick-up (14,000) birds at day 53. This activity in the shed meant that there could have been some means of cross contamination across the shed categories used in this study. Nevertheless, a pattern similar to that seen at Donnybrook occurred at the last two sampling dates (with a latter being a few hours after the final pick up).

On day 49, the lowest levels were found in the "new" N1 litter, as was seen in the Donnybrook study. From this second last sampling to the last sampling, there was a marked reduction in both "re-use" litters (R1 and R2) and in the N2 "new" litter (a similar pattern as seen at the Donnybrook farm). As with the Donnybrook farm, the highest count on the final sampling day at the Laravale farm was the N1 "new" litter at 2,300 MPN/g.

Levels of **Clostridium perfringens** *at both Donnybrook and Laravale through the chicken production cycle*

Figures 2.56 and 2.57 show the levels of *Clostridium perfringens* through the cycle at Donnybrook and Laravale respectively. For both farms, *Cl. perfringens* levels were higher in the "re-use" litter than the "new" litter prior to the placement of chickens. Indeed, only the N2 "new" litter for the Donnybrook farm had detectable levels of this organism, with the N1 sample from this farm and both N1 and N2 samples at the Laravale farm not containing detectable levels of *Cl. perfringens.* Prior to chicken placement, the "re-use" litter at the Donnybrook farm had noticeably higher levels of *Cl. perfringens* (6,400 and 8,500 CFU/g) than the "re-use" litter at the Laravale farm.

At both farms, the *Cl. perfringens* levels stayed around the level of 10^3 to 10^4 CFU/g for the length of the cycle with one exception. At the Laravale farm, there was a noticeable peak in the *Cl. perfringens* levels in both "new" litter samples at day 7.

Figure 2.48 Levels of *Escherichia coli* recovered from litter at brooder (N1 and N2) grow-out (R1 and R2) ends of shed over a broiler cycle (Donnybrook May - July 2007). All results for the environmental sample were below the detection limit. At week 2 the R2 Sample was also below the detection limit.

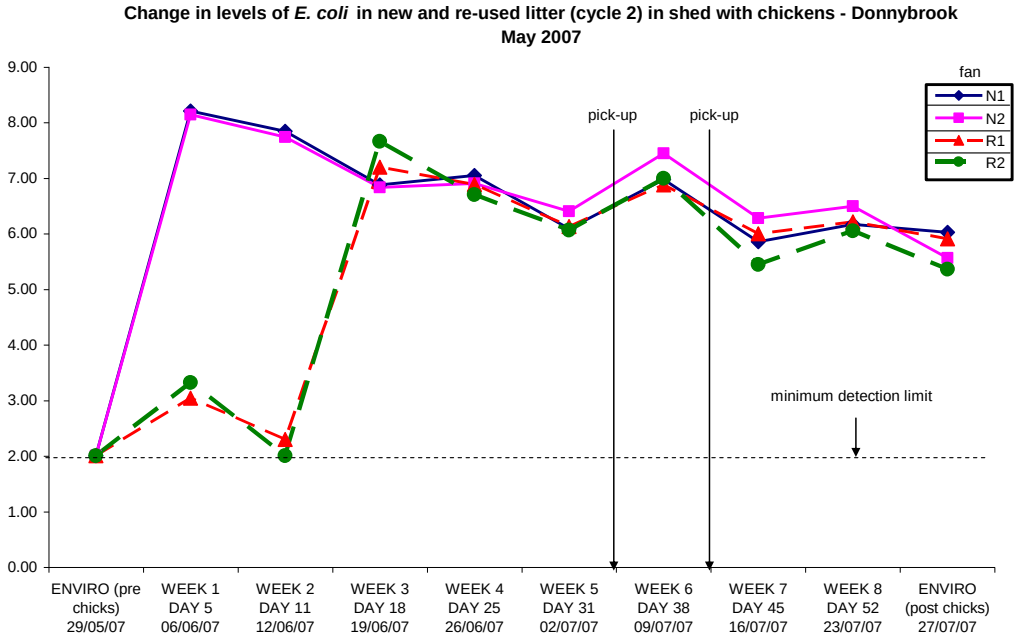


Figure 2.49 Levels of *Escherichia coli* recovered from litter at brooder (N1 and N2) grow-out (R1 and R2) ends of shed over a broiler cycle (Laravale August - October 2007)

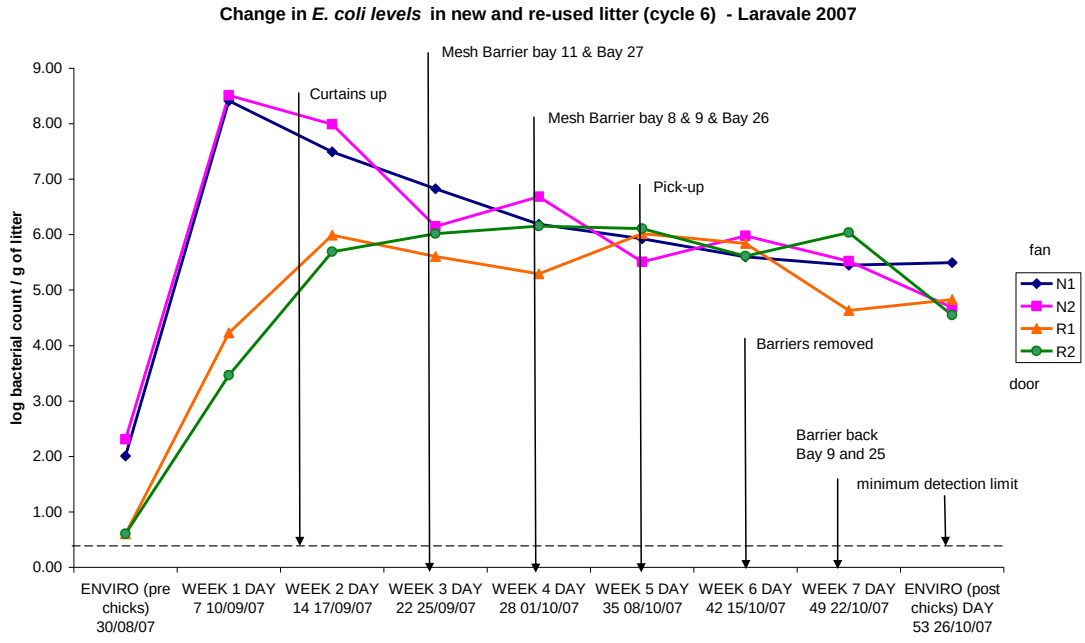
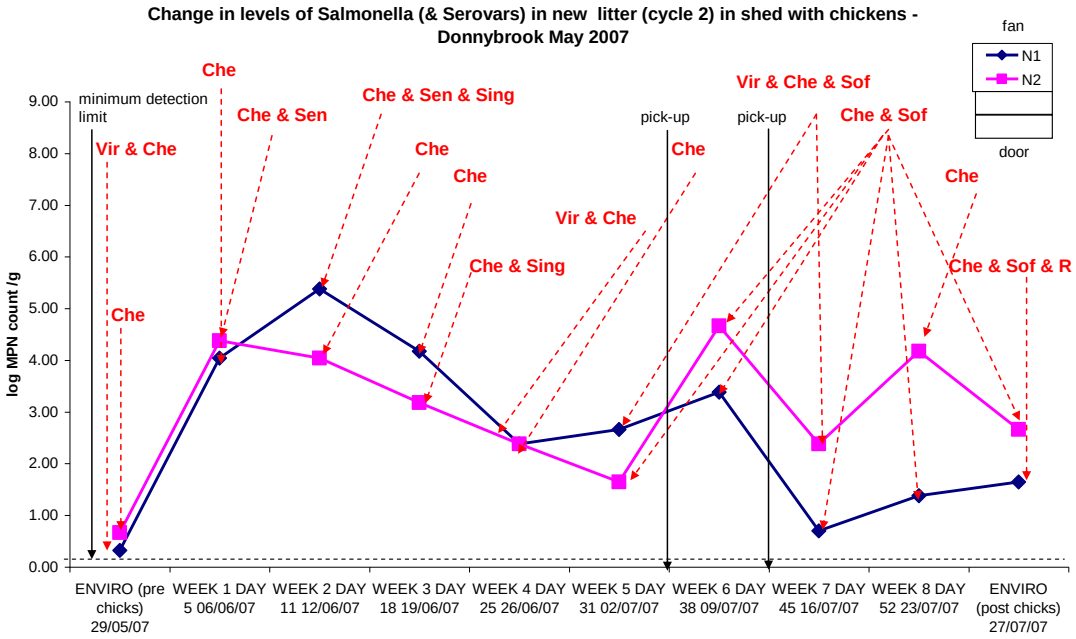
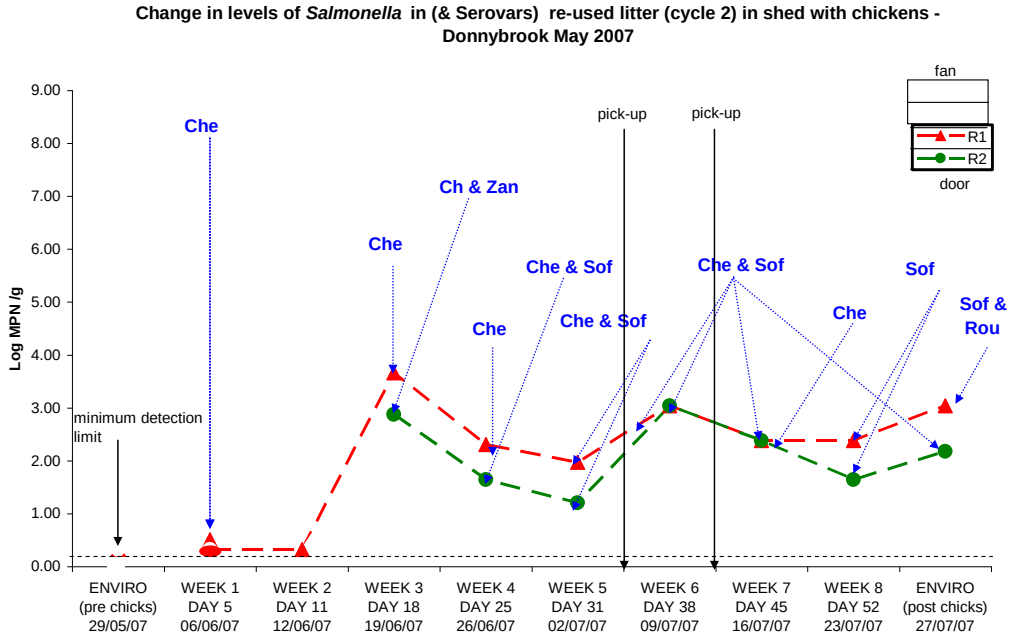


Figure 2.50 Levels of *Salmonella* and serovars recovered from litter at brooder end (N1 and N2) of shed over a broiler cycle (Donnybrook May - July 2007)



Abbreviations: Che Chester; R - Rough Sen – Seneftenburg; Sing – Singapore; Sof – Sofia; Vir – Virchow.

Figure 2.51 Levels of *Salmonella* and serovars recovered from litter at grow-out end of shed over a broiler cycle (Donnybrook May - July 2007)



Abbreviations: Che Chester; R - Rough; Sof – Sofia; Zan - Zanzibar

Figure 2.52 Levels of *Salmonella* and serovars recovered from litter at brooder end of shed over a broiler cycle (Laravale August - October 2007)

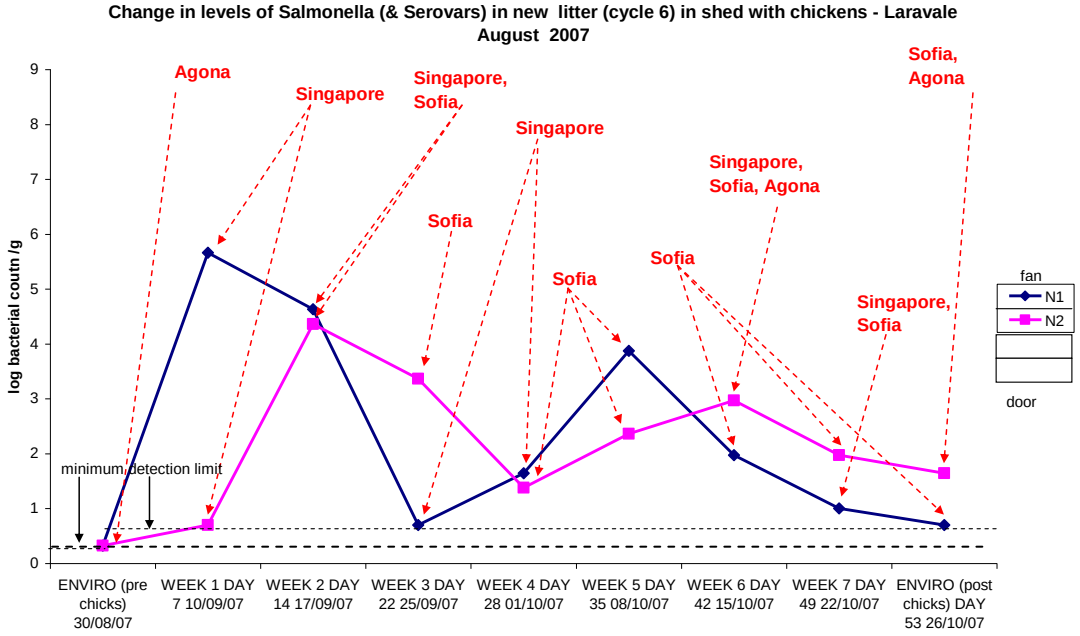


Figure 2.53 Levels of *Salmonella* and serovars recovered from litter at grow-out end of shed over a broiler cycle (Laravale August - October 2007)

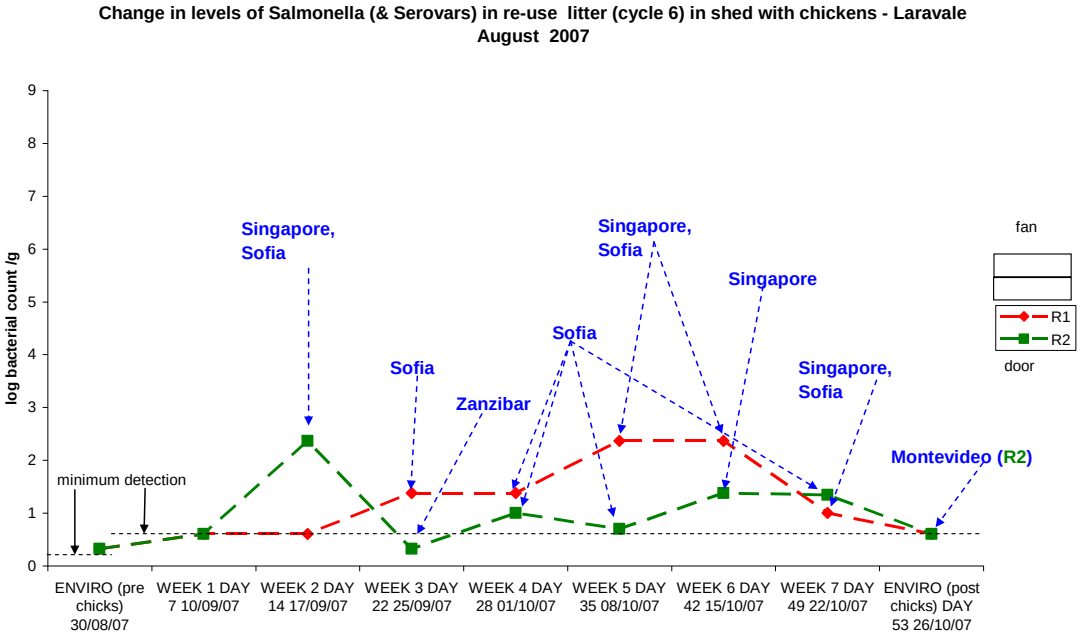


Figure 2.54 Levels of *Campylobacter* recovered from litter at brooder (N1 and N2) grow-out (R1 and R2) ends of shed over a broiler cycle (Donnybrook May - July 2007)

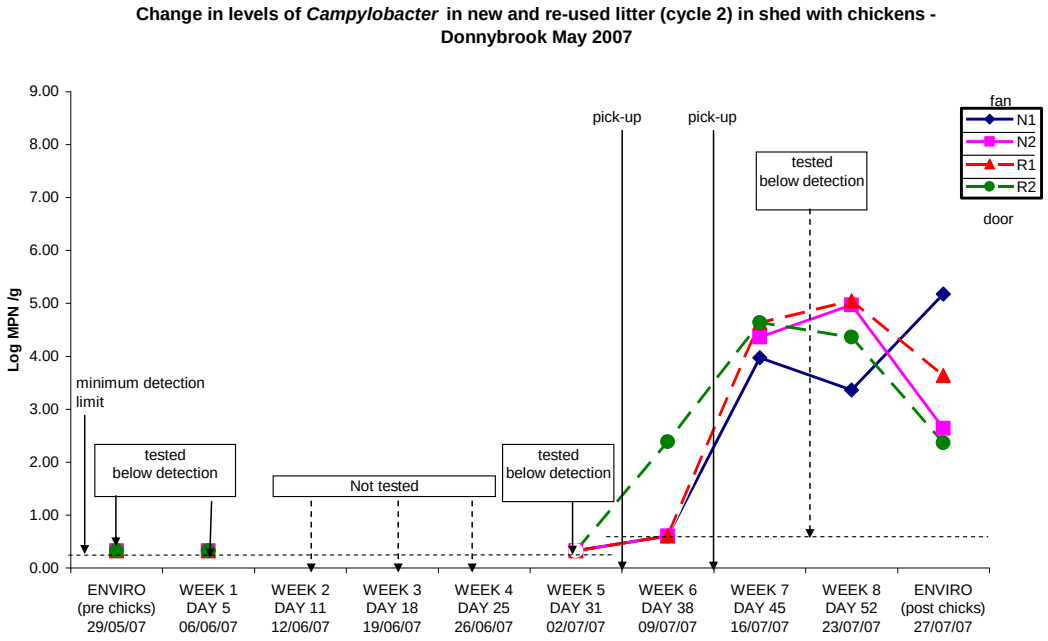


Figure 2.55 Levels of *Campylobacter* recovered from litter at brooder (N1 and N2) grow-out (R1 and R2) ends of shed over a broiler cycle (Laravale August - October 2007)

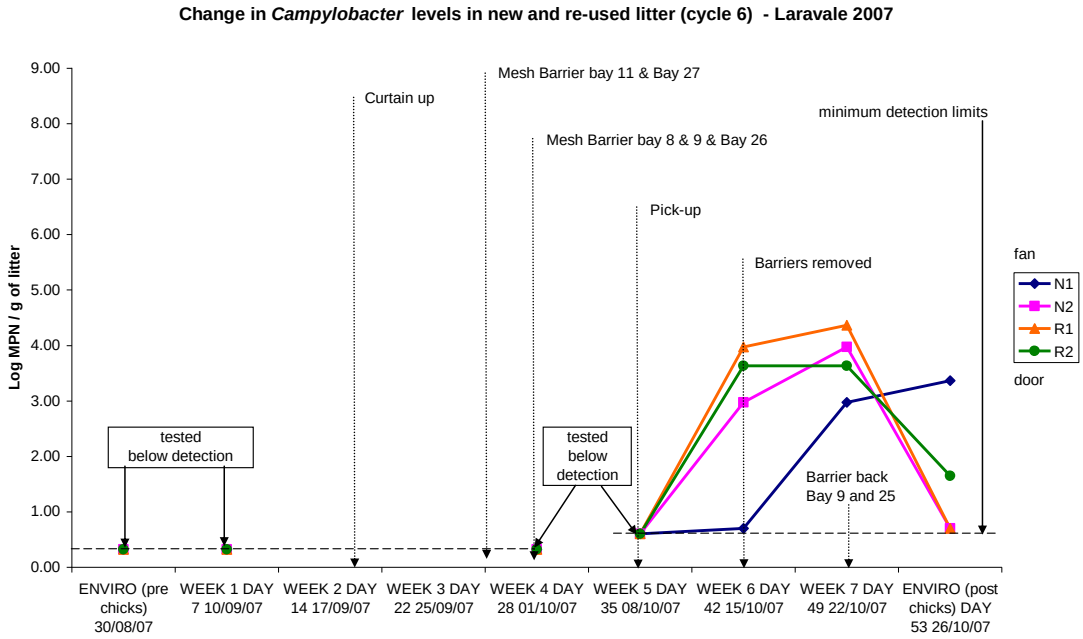


Figure 2.56 Levels of *Clostridium perfringens* recovered from litter at brooder (N1 and N2) grow-out (R1 and R2) ends of shed over a broiler cycle (Donnybrook May - July 2007)

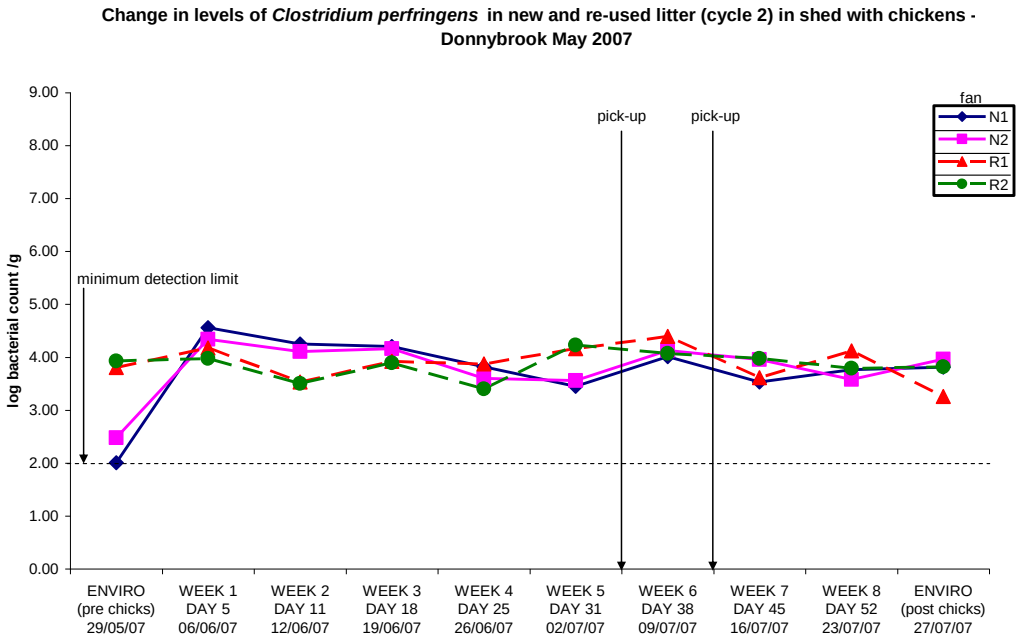
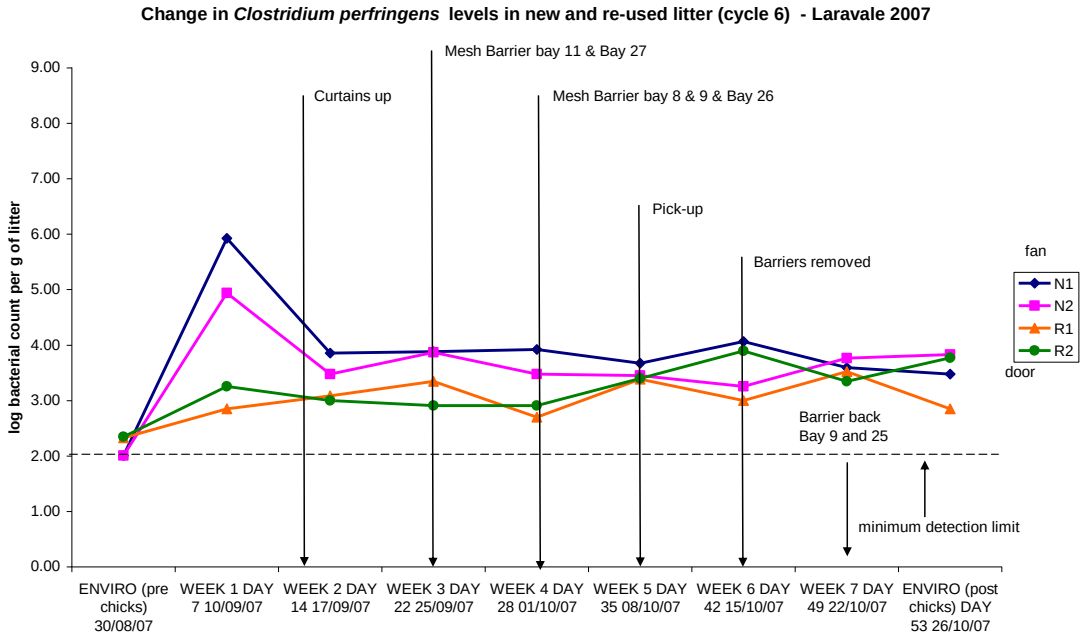


Figure 2.57 Levels of *Clostridium perfringens* recovered from litter at brooder (N1 and N2) grow-out (R1 and R2) ends of shed over a broiler cycle (Laravale August - October 2007)



a_W *levels in litter at both Donnybrook and Laravale through the chicken production cycle*

Figures 2.58 and 2.59 show the water activity levels recorded through the chicken production cycle at the Donnybrook and Laravale farm respectively.

As noted earlier, the litter management practices adopted at the two farms varied. The "re-used" litter at the Laravale farm contained a mixture of much older litter – coming from litter push-up cycle 5 after five broiler cycles. At Donnybrook, the "re-used" litter had been through only a single litter push-up cycle.

This difference in litter history (cycle 6 litter) may explain why the "reuse" litter at Laravale (prior to chicken placement has a much lower water activity level (0.76 and 0.80) than the "re-use" litter at Donnybrook prior to placement (0.92 and 0.93). Indeed, the level seen at the Laravale is in the range that is region that is regarded as not being supportive for the growth of *Salmonella* (0.75 – 0.80) while at Donnybrook the level is a region (0.90 – 0.95) that is regard as being supportive of *Salmonella* growth (Carr et al. 1994)

The "re-use" litter at Laravale stayed in the non-growth supportive range at the day 7 and 14 samplings. The "re-use" litter at Donnybrook showed a drop and was in the non-growth supportive range at days 11 and 18.

The "new" litter at Laravale had a very low water activity prior to chicken placement, although high levels (0.87 and 0.93) were recorded at day 7 in the presence of the chickens. At the time of the first pickup at Laravale (day 35, Figure 2.59), there was a spike in water activity levels for both "re-use" and "new" litter to levels above >1, a level that is reportedly beyond the zone for the growth of *Salmonella* (Carr et al. 1994)

Overall, at both farms, there was a general tendency for the water activity levels to be lower in the "re-used" litter (R1 and R2) than in the "new" litter (N1 and N2). Further, the water activity results indicate that different ends of the shed (and therefore the different litter types) support slightly different environments that could have an impact on the overall bacterial growth.

Figure 2.58 aW levels recorded in litter at both the brooder end (N) and grow-out end (R) over a full broiler cycle (Donnybrook May-July 2007)

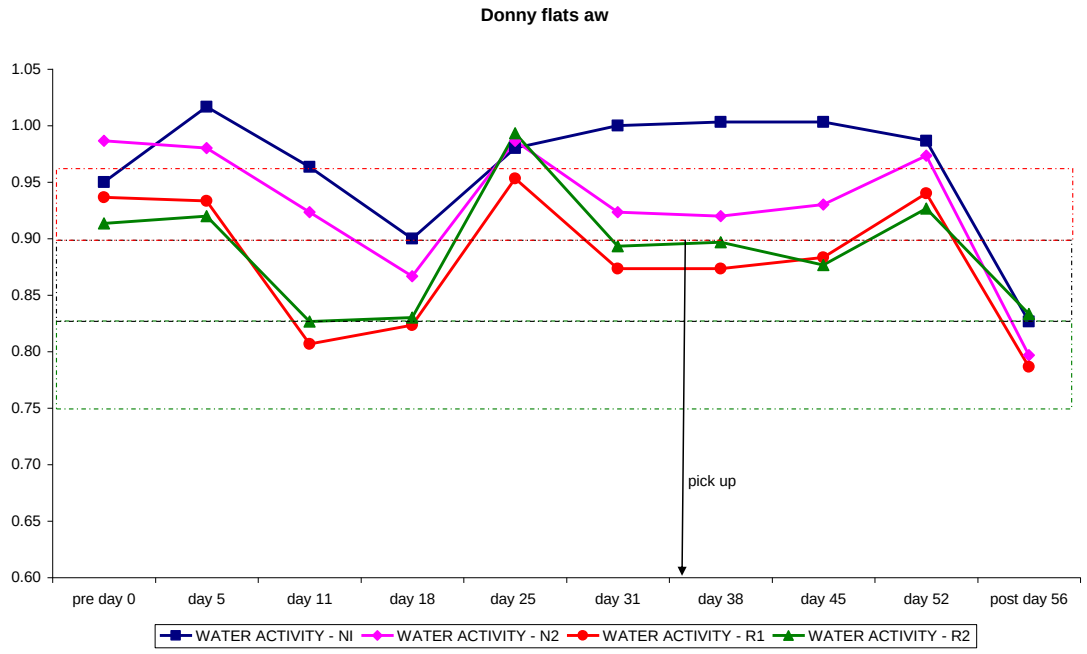
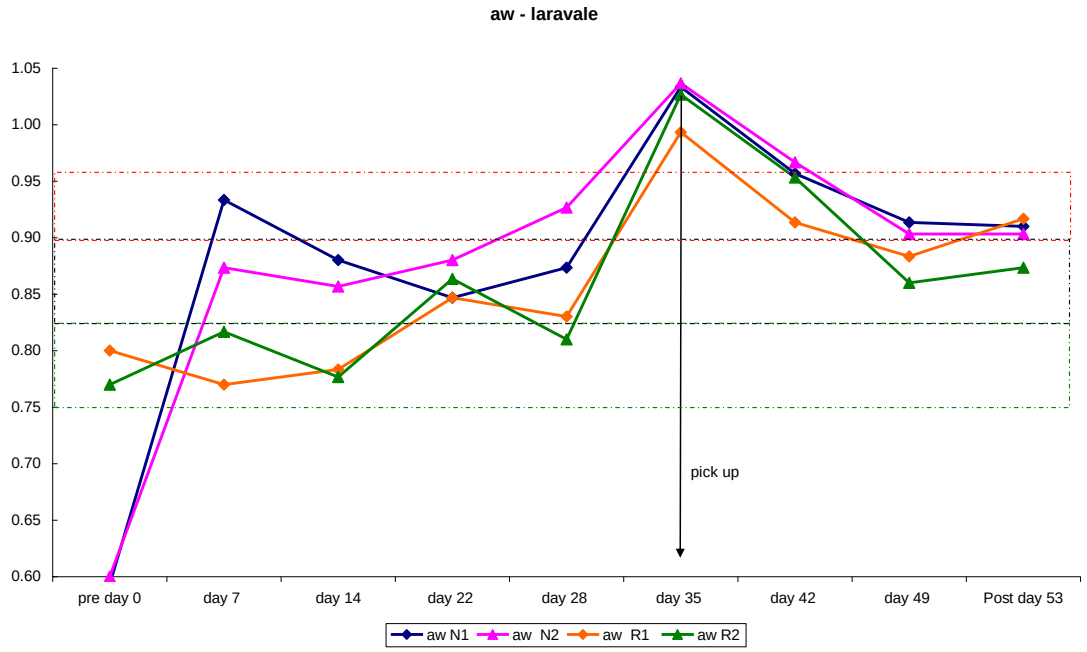


Figure 2.59 aW levels recorded in litter at both the brooder end (N) and grow-out end (R) over a full broiler cycle (Laravale August – October 2007)



aW *and moisture levels in litter at both Donnybrook and Laravale through the chicken production cycle*

Figures 2.60 and 2.61 show the aW and moisture levels recorded through the chicken production cycle at the Donnybrook and Laravale farm respectively.

As was noted in the earlier pile studies, there does not appear to be a distinct linear relationship between the moisture content (the bound - non available plus the free available water for microbial activity) and the aW (the free available water for microbial activity) levels.

Prior to chicken placement, both moisture content and aW levels indicate that the "new" litter at Laravale is much drier than that at Donnybrook.

At Donnybrook, following the introduction of the chickens, the moisture content in both "new" and the "re-use" litter was around 25% for the full cycle. However, there was a difference between the aW levels of the two litter types.

As an overall comment for the majority of the time the re-used end had lower water activity levels (between 0.83 – 9.00) than the new end (levels of 0.93 – 1.00).

At Laravale, following the introduction of the chickens, the moisture content fluctuated around the 25% level for the full production cycle. Until, the week of the first pick up, the aW levels in the "new" litter were in the range 0.85 – 0.90 while the "re-use" litter showed levels 0.78 – 0.83.

Figure 2.60 aW and moisture content levels recorded in litter at both the brooder end (N) and grow-out end (R) over a full broiler cycle (Donnybrook May-July 2007)

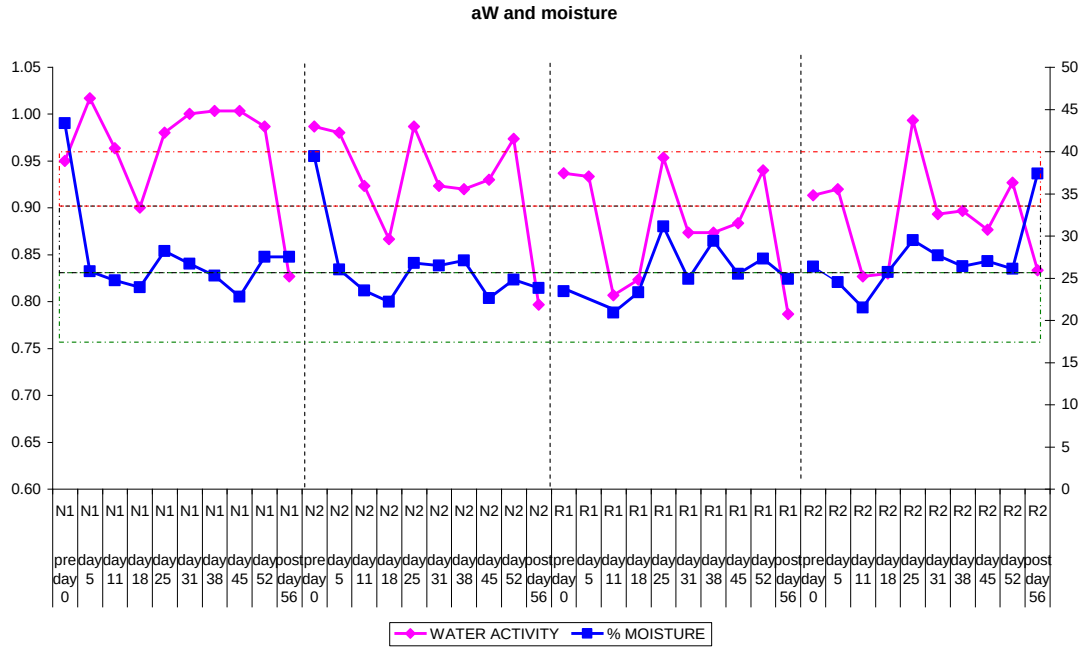
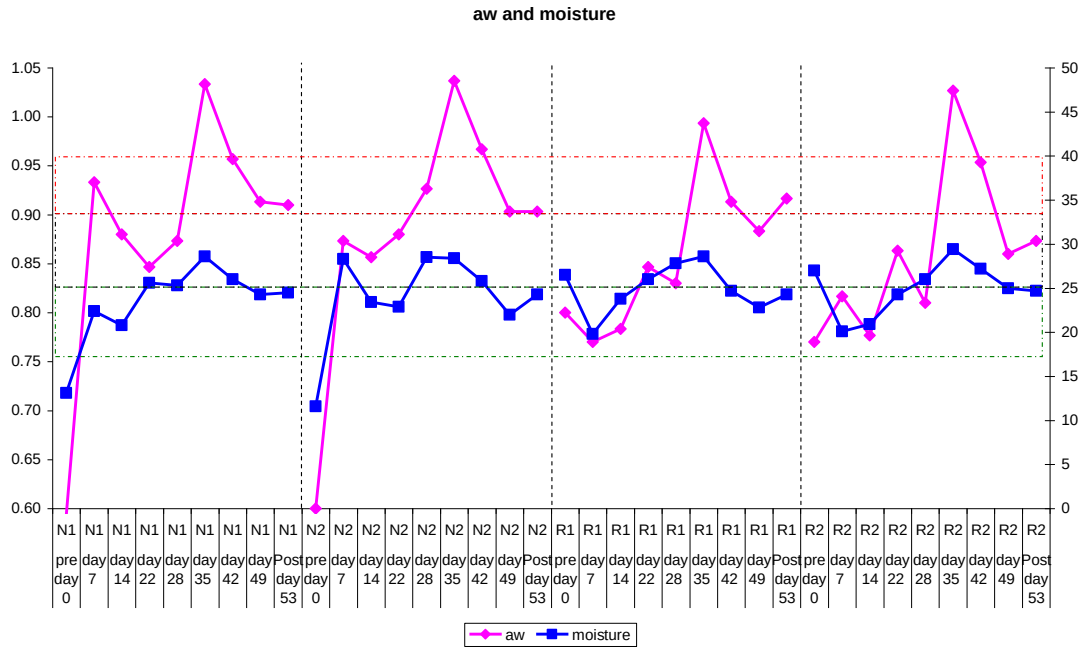


Figure 2.61 aW levels recorded in litter at both the brooder end (N) and grow-out end (R) over a full broiler cycle (Laravale August – October 2007)



pH levels in litter at both Donnybrook and Laravale through the chicken production cycle

Figures 2.62 and 2.63 show the litter pH levels recorded through the chicken production cycle at the Donnybrook and Laravale farm respectively.

At both farms, the "re-use" litter had higher pH than the "new" litter prior to chicken placement, with this difference remaining till around day 14. The "re-use" litter at Laravale had a pH of around 8 till day 14, while the "re-use" litter at Donnybrook was slightly higher (around pH 8.5) and stayed at this level until about day 14.

The "new" litter at Donnybrook had pH levels of around 7 to 8 until about day 31. At Laravale, the "new" litter had a much lower initial pH (6.5 and 7.0), although the pH levels rose to 8.5 at day 14 and even to around 9 at day 28.

From day 35 onwards, the pH levels in both litter types ("new" and "re-use") at both farms were generally in the same range of 8 – 8.5.

On both farms, at the last reading, after the final chicken pick-up, the middle sections of the shed (the N2 "new" and the R1 "re-use") litter showed slightly higher pH levels than the two extreme ends of the shed (the N1 "new" and the R2 "re-use"). It is possible that the more confined nature of the middle section of the shed as compared with the more open nature of the ends (doors, fans and so on) may have affected the evaporation of ammonia and hence the pH levels.

Figure 2.62 pH levels recorded in litter at both the brooder end (N) and grow-out end (R) over a full broiler cycle (Donnybrook May-July 2007)

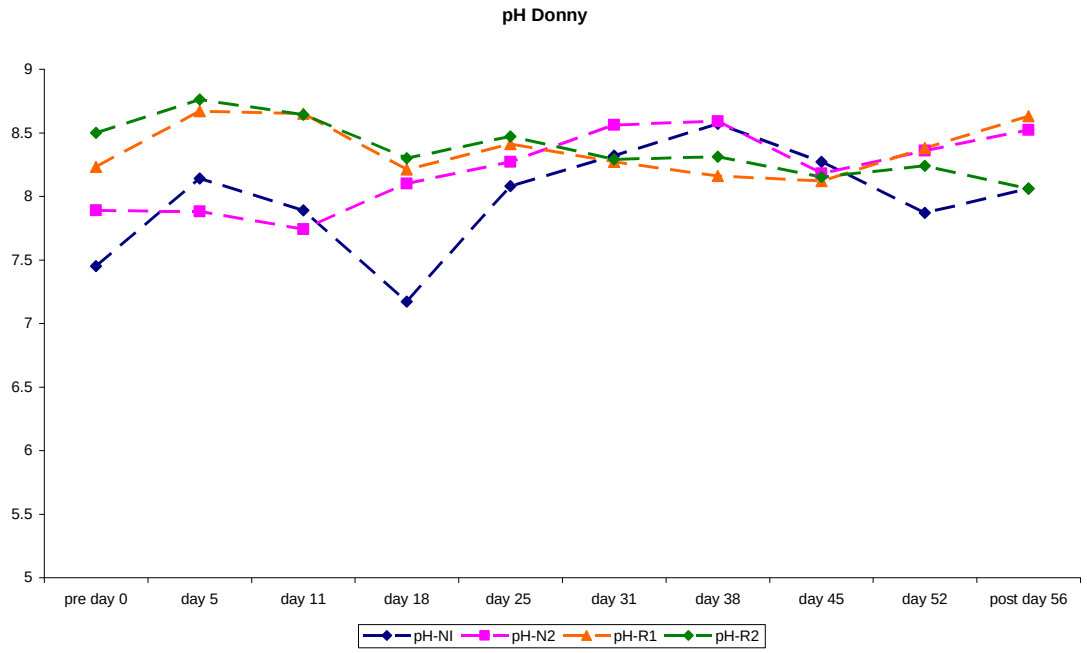
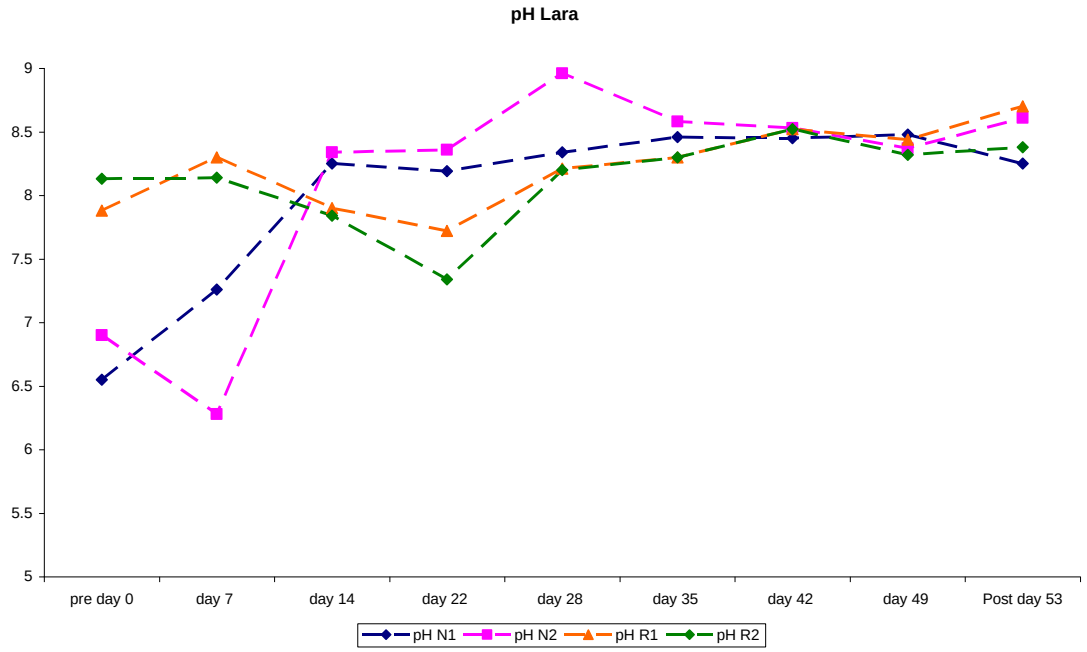


Figure 2.63 pH levels recorded in litter at both the brooder end (N) and grow-out end (R) over a full broiler cycle (Laravale August – October 2007)



Litter temperatures at Donnybrook through the chicken production cycle

The temperatures for the surface litter before chicken placement as well as on days 18, 25, 38, 42 and 52 were continuously monitored for 24 hours from the day of sampling through to the next morning at the Donnybrook farm. Figure 2.64 shows the results of this monitoring.

On the day of placement the four "re-use" litter probes recorded temperatures in the range of 25 to 35ºC, warmer than the "new" litter temperatures which ranged from 20 – 25ºC. Neither litter type showed any marked cooling down during the night, a feature which was shown by the ambient probe.

On day 18, there was little difference in temperatures recorded in the two litter types. The high spikes probably indicate chicken activity in the vicinity of the probes. The litter temperatures were slightly higher than the ambient temperature over the 24 hours.

On day 25 the temperatures in the "new" and the "re-use" litter were not distinguishable. The spiking is now much greater than at day 18, indicating more chicken activity as would be expected as the chickens grow. The litter temperature (25 to 35ºC) was stable over both day and night while the ambient probe a very stable 20ºC.

By day 38 the first pick-up had occurred. There was a trend for more chicken activity – as shown by more spikes – in the shed area with "re use" litter as compared with the "new" litter. The litter temperature range was between 25 to 35ºC for both litter types.

By day 42, the four temperature probes in the "re-use" litter showed considerable spikes – indicating chicken activity – over the full recording period. In contrast, the probes in the "new" litter showed this spiking activity mainly during the night and early morning. The range in both litter types remained the same as day 38 - 25 to 35ºC.

By day 52, there are few spikes in the probes in either litter type – indicating far less chicken activity. This general quietness in the shed was also noted in the field observation log (see Appendix 1).

Litter temperatures at Laravale through the chicken production cycle

This trial occurred from late August till early October while the Donnybrook trial covered the period from June to July. Similar days of the cycle were studied (days 7, 14, 28, 42 and 49) as in the Donnybrook study. Figure 2.65 shows the results of the litter temperature monitoring.

On the day of placement, all probes in the "reuse" showed temperatures above those in the "new" litter. Indeed, three of the four "re-use" litter probes showed temperatures in the range of 23 to 25ºC while the "new" litter probes generally showed temperatures of < 20ºC. Over the night period, all litter probes recorded temperatures above the ambient probe.

On Day 7, as expected there were spikes in temperature, due to chicken activity, in the "new" litter areas. Both the "new" and the "re-use" litter temperatures were in the same range - 25 to 30ºC.

On day 14, there were noticeable spikes in the "new" litter temperatures recorded by some of the probes. In contrast, the probes located in the "re-use" section of the shed showed no such indication of chicken activity and showed a stable pattern in the range of 28 to 30ºC.

On days 28, 42 and 49, there were similar amounts of spiking occurring in the "new" and the "re-use" litter probes. At all three days, the litter probes showed temperatures in the range of 25 to 35ºC.

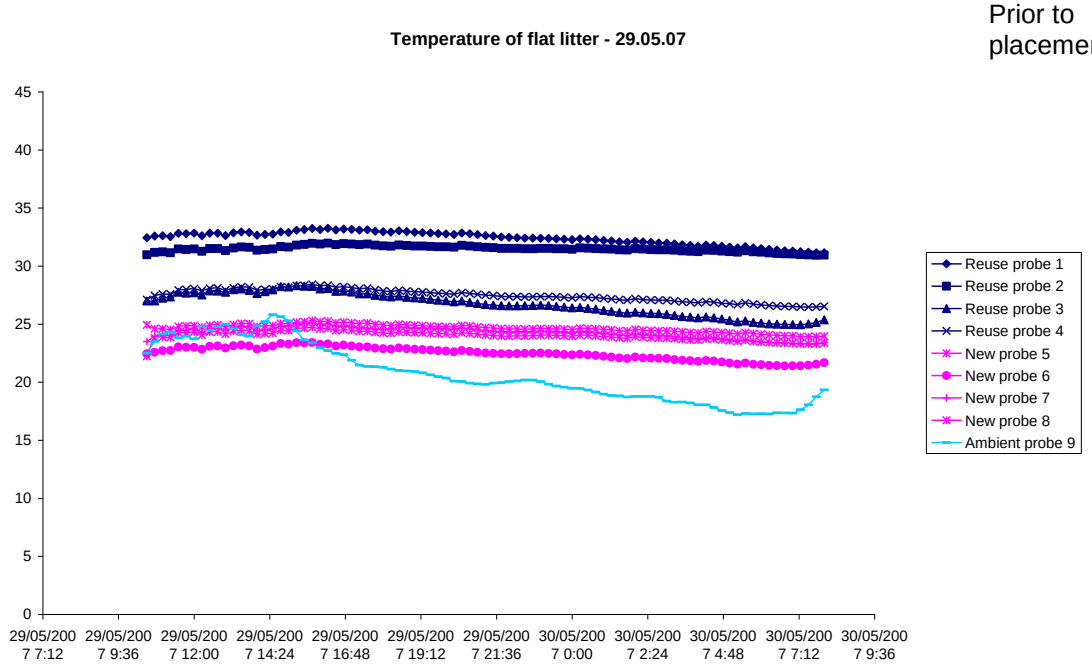
Overall Comments on Litter Temperatures

Overall, the temperatures in both "new" and "re-use" litter showed similar patterns at both farms. At both farms, the "re-use" litter was warmer (due to microbial activity) than the "new" litter prior to chicken placement. At both farms, the litter temperatures were in a range that would be suitable for both *Campylobacter* and *Salmonella*.

Figure 2.64. Litter temperatures recorded in brooder end (New) and grow-out end (Reuse) and ambient air temperature over a 24 hour period at various time points in the broiler cycle (Donnybrook June – July 2007). A) Prior to chick placement; B) Day 18; C) Day 25; D) Day 38; E) Day 45; F) Day 52.

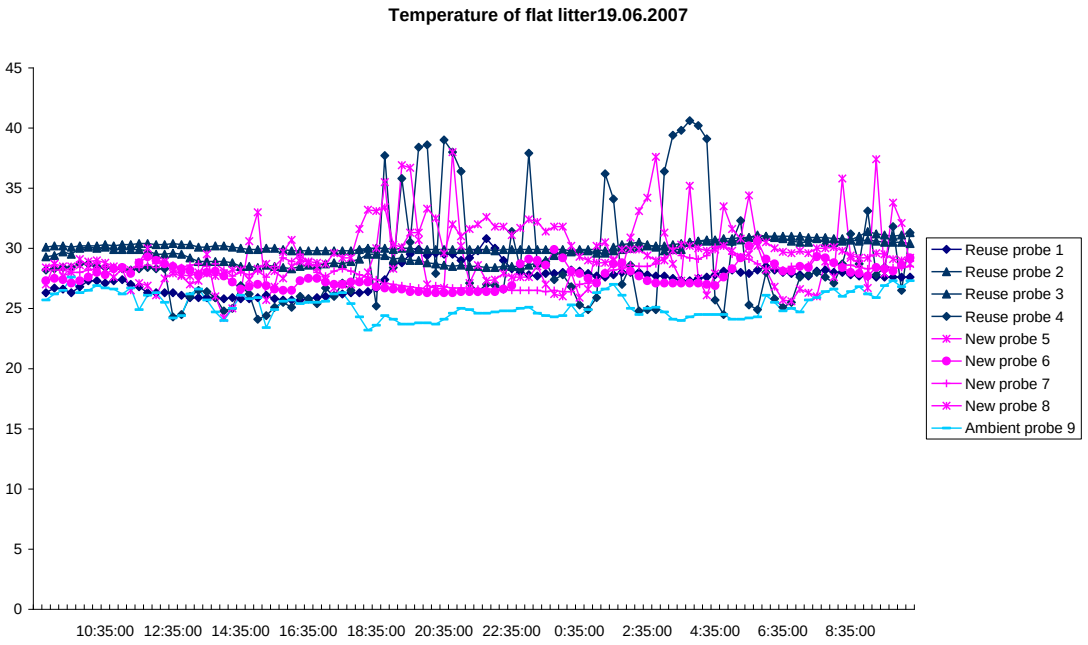
A.

Prior to placement



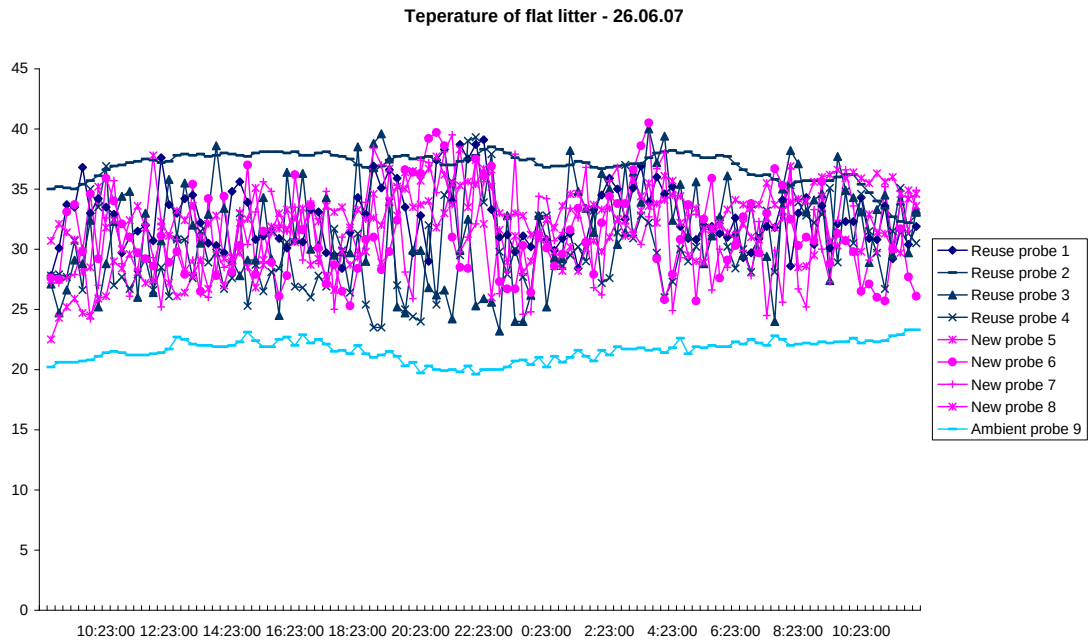
B.

Day 18



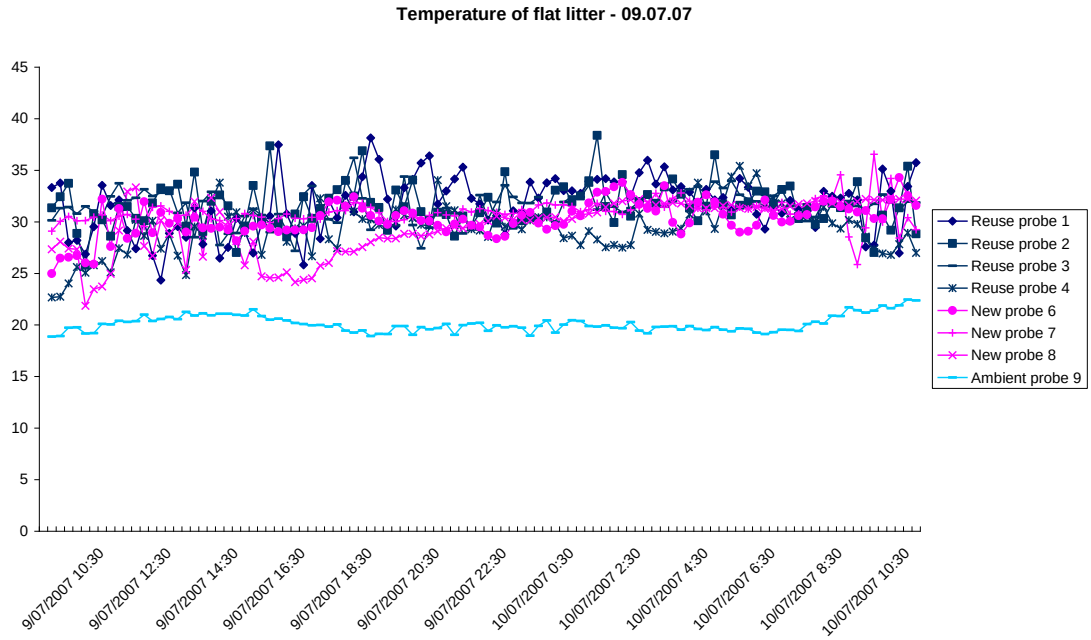
C.

Day 25

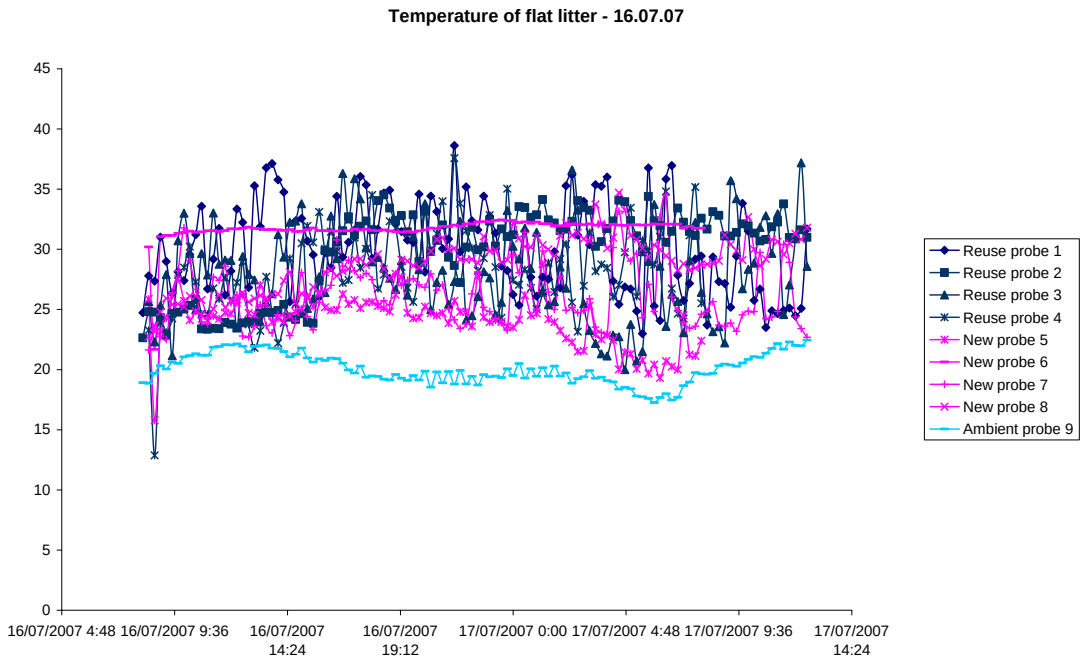


D.

Day 38



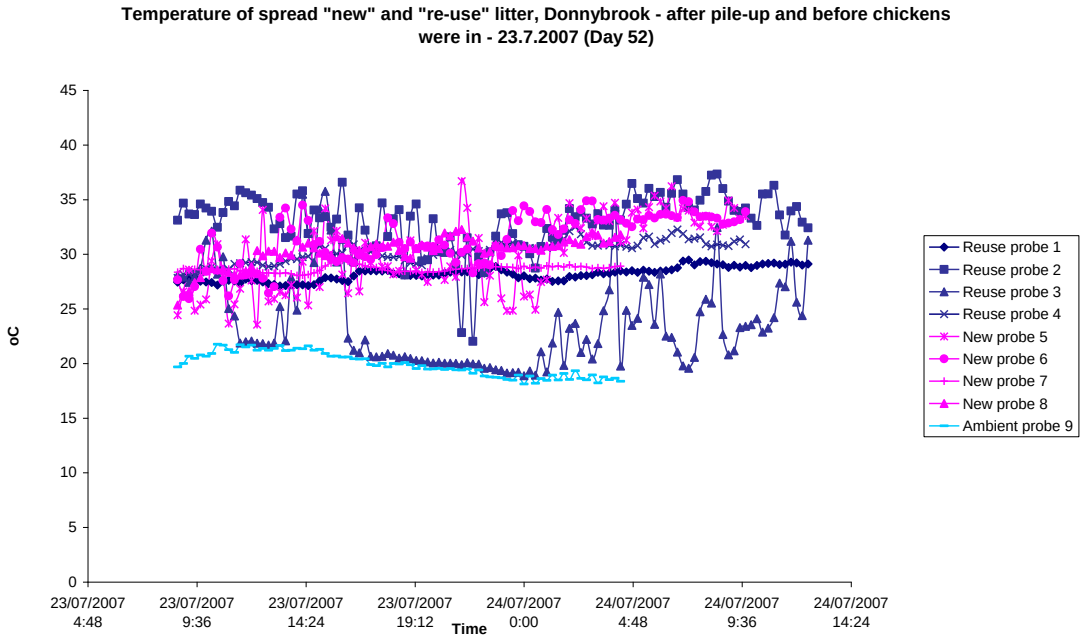
E.



Day 42

F.

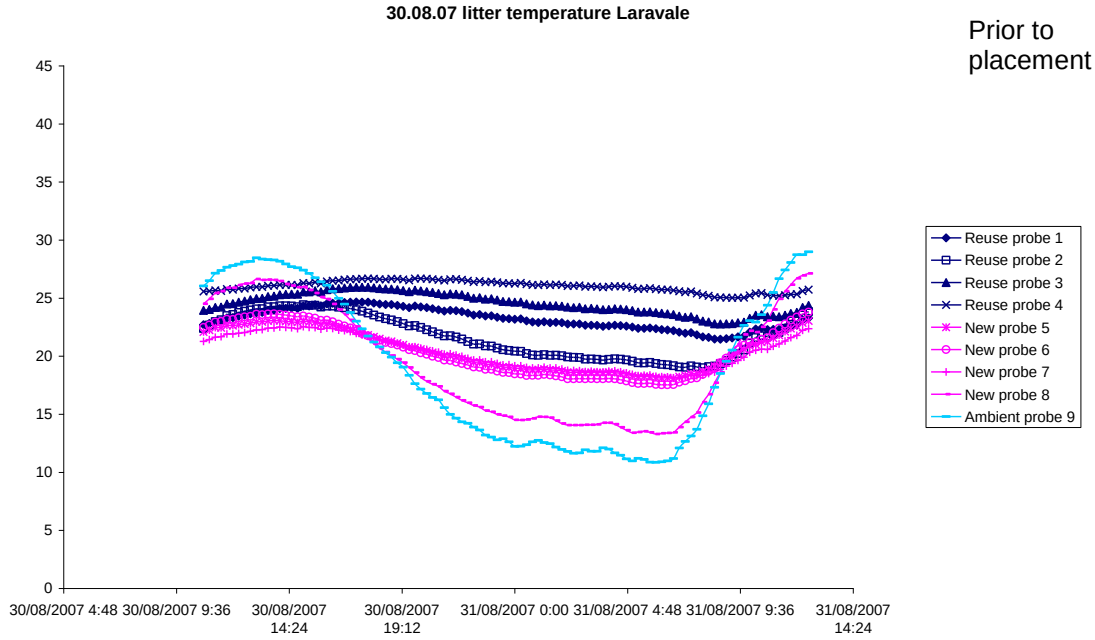
Prior to placement



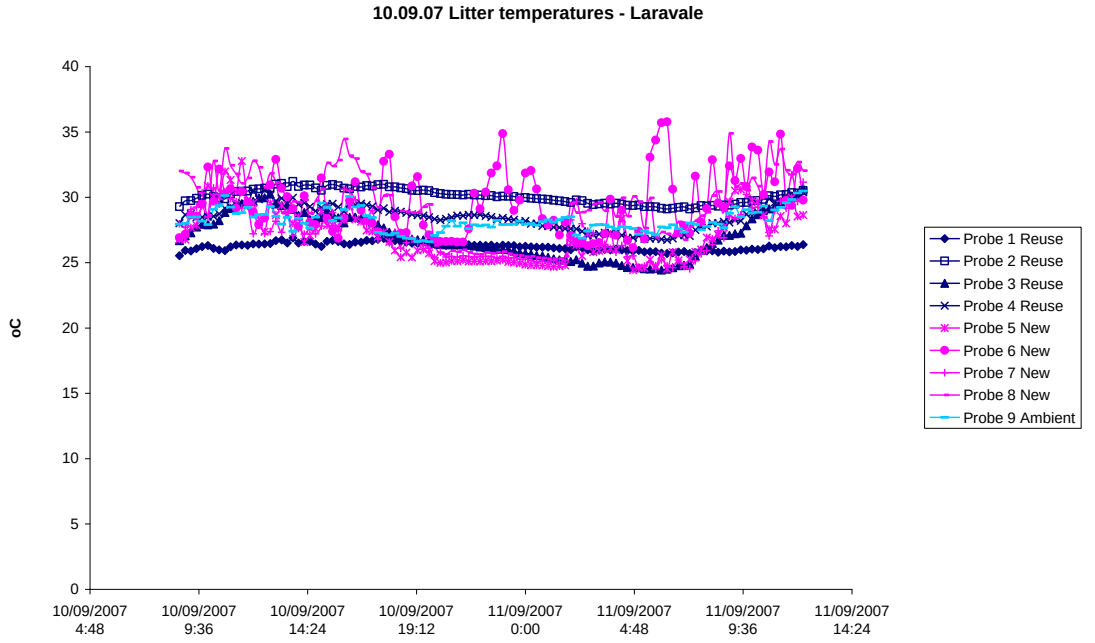
Day 52

Figure 2.65 Litter temperatures recorded in brooder end (New) and grow-out end (Reuse) and ambient air temperature over a 24 hour period at various time points in the broiler cycle (Laravale August – October 2007). A) Prior to chick placement; B) Day 7; C) Day 14; D) Day 28; E) Day 42; F) Day 49

A.

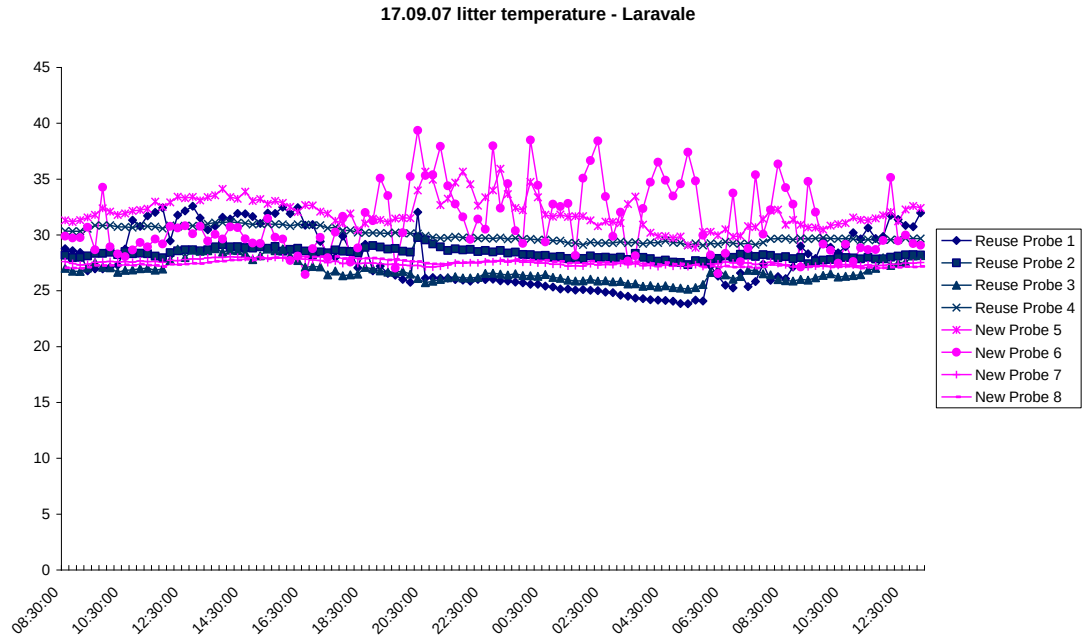


B.



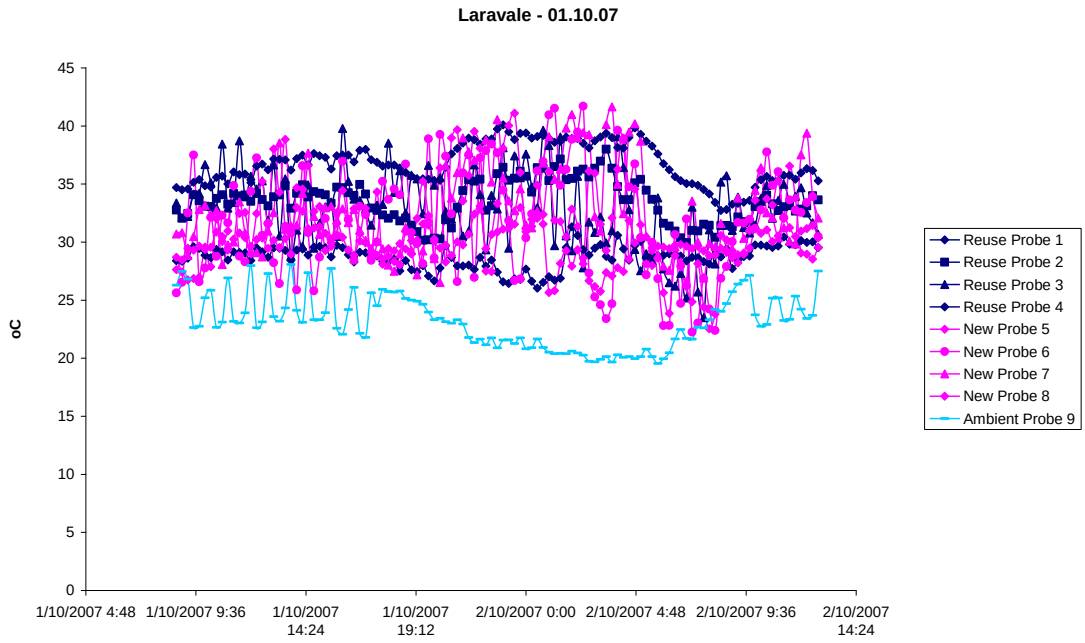
C.

Day 14



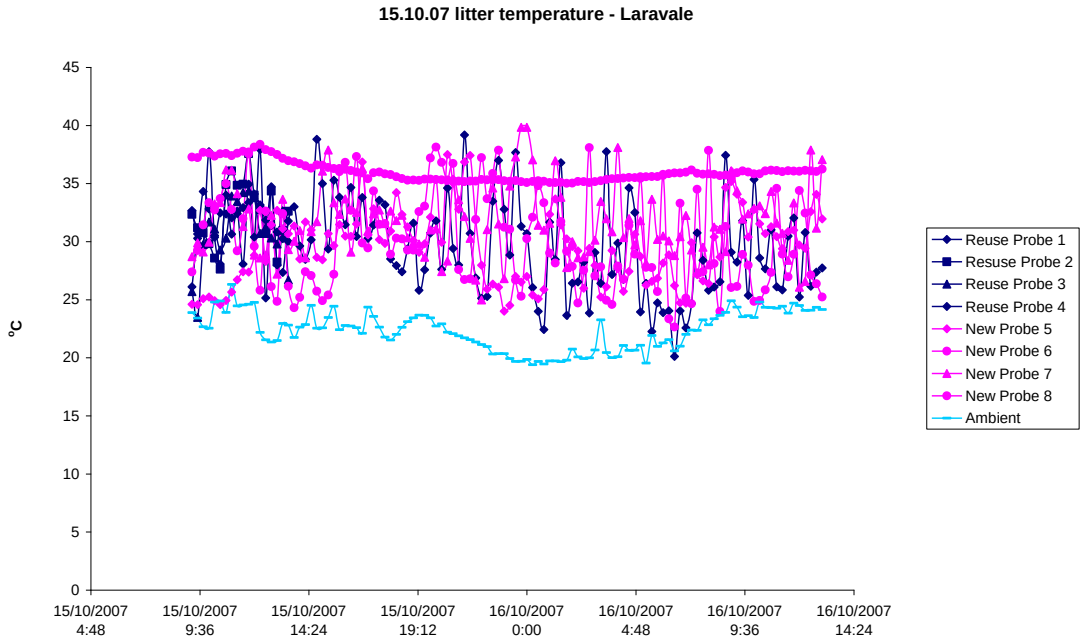
D.

Day 28



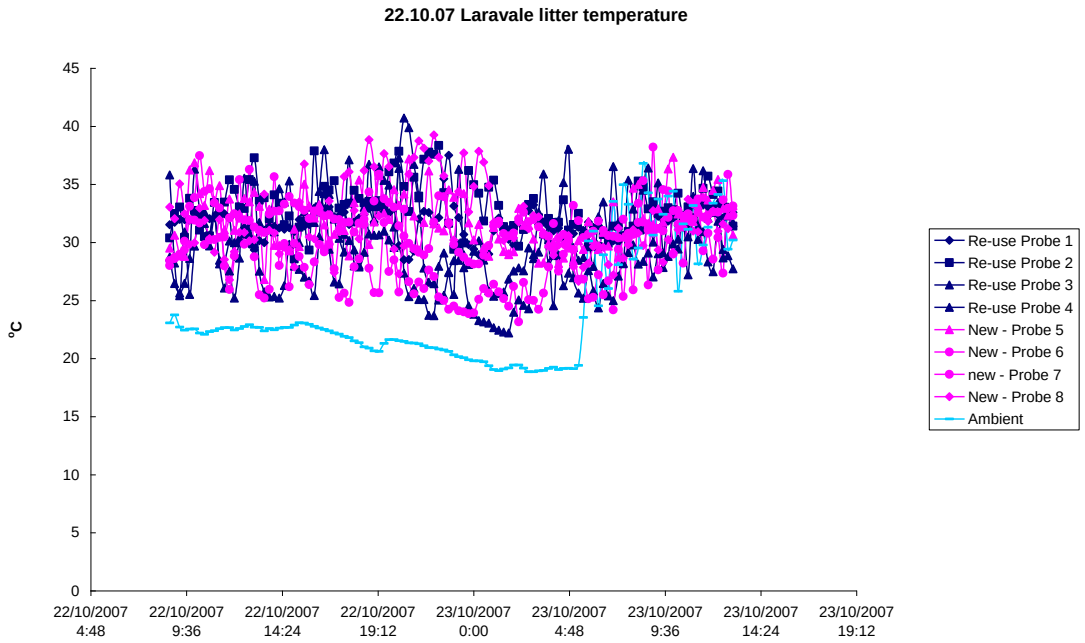
E.

Day 42



F.

Day 49



Litter depths recorded at Donnybrook and Laravale through the chicken production cycle

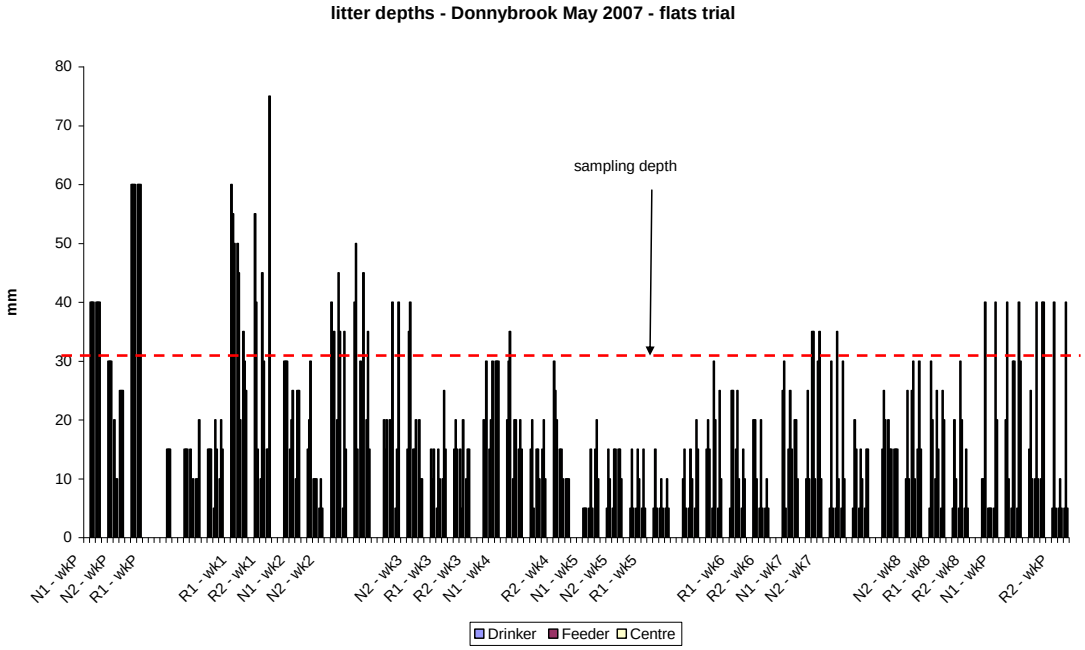
Figures 2.66 and 2.67 illustrate the litter depths as measured over a chicken production cycle at the Donnybrook and Laravale farm respectively. Within each of the three bays that had been randomly selected for sampling, the litter depth was measured at three locations – around the drinker line, around the feeder line and in the centre of the shed (the latter being away from both feeder and drinker lines). At each particular site (i.e. at the drinker line or feeder line or the centre), three depth measurements were taken. Figures 2.66 and 2.67 also show the depth used for the biological sampling (i.e. 30 mm depth).

Overall, across both Farms, the litter depth around the drinkers tended to decrease over the cycle, possibly associated with water spillage. The field notes (Appendix 1 – Field observations) noted that the water lines were leaking on days 31, 38 and 45.

At Laravale, litter depths of around 30 -40 mm were maintained in the centre and feeder line areas across the production cycle (Figure 2.67). At Donnybrook, similar depths were maintained in the feeder line area but there was tendency for the depth in the centre area to drop over the early part of the cycle and thus for the litter depth to be lower than that seen at Laravale.

Figure 2.66 Litter depths (A) across the different sampling days and (B) categorized as "drinker", "feeder" and "centre" for Donnybrook (June – July 2007)

A.



B.

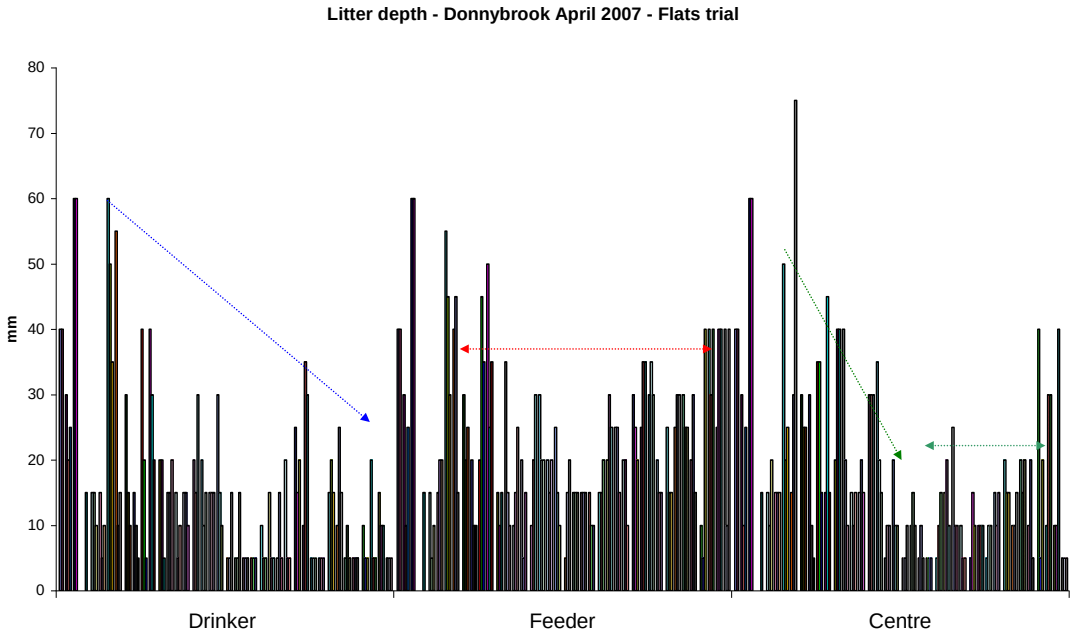
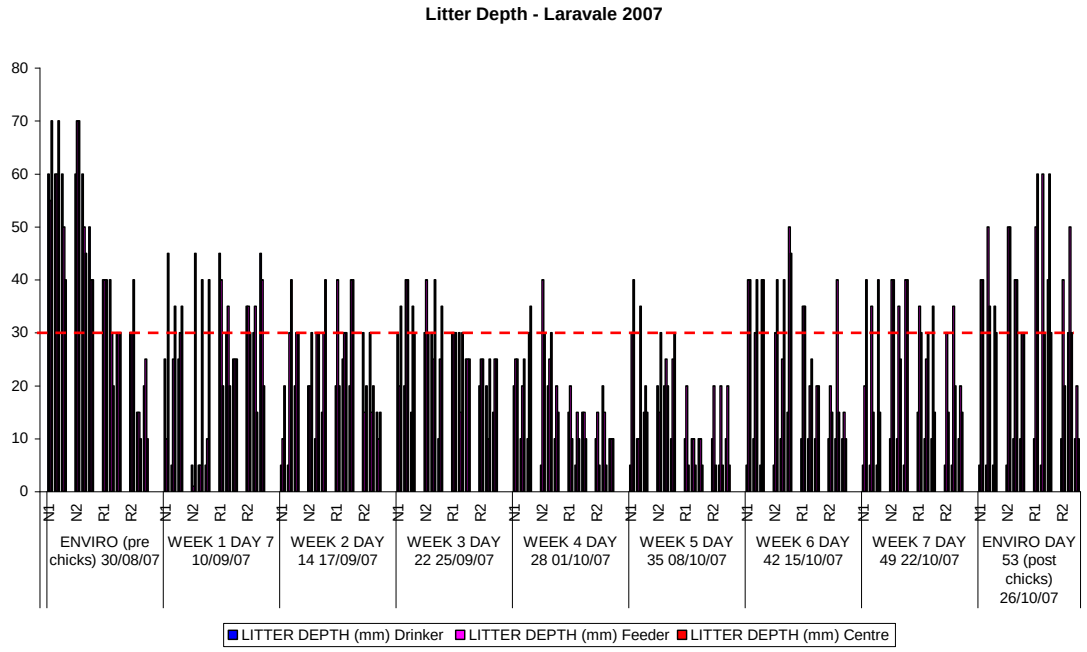
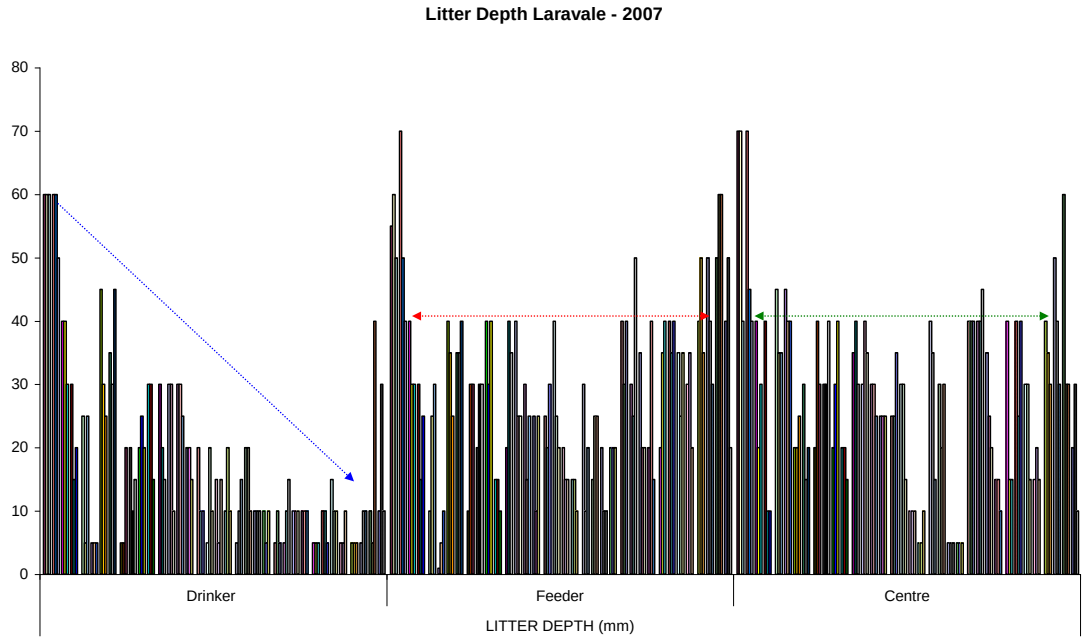


Figure 2.67 Litter temperatures depths (A) across the different sampling days and (B) categorized as “drinker”, “feeder” and “centre” for Laravale (August – October 2007).

A.



B.



Discussion

Litter and food-borne pathogen survival

Litter once treated (piled) and re-used (through a chicken cycle) in the shed can have varying physical and microbiological parameters, resulting in the litter being either supportive or inhibitory to pathogens. The present study has demonstrated that both *Salmonella* and *Campylobacter* can persist in litter through various stages of the chicken cycle. There are a range of survival studies in literature that help provide some basis of knowledge. Studies carried out under laboratory conditions have shown *Salmonella* to survive in poultry feed and litter at 25ºC for 16-18 months and at 38ºC for 40 days in feed and 13 days in litter (Williams and Benson 1978) indicating the impact of temperature on survival. *Campylobacter* levels in litter declined from 2-9 x 10^6 cfu/g to <5 cfu/g in 72 hours on the removal of artificially contaminated chicks (Shanker et al. 1990) showing a poor survival potential in litter following excretion from the chick. *Campylobacter* is known to be susceptible to environmental stress (Jones 2001) whilst *Salmonella* has been shown to persist even within dusty environments in poultry sheds (Chinivasagam et al. 2008, Bhatia et al. 1979). Covering the pile or the addition of water to the pile both resulted in the lowering of both aerobic and anaerobic bacterial levels (Macklin et al. 2006). *Salmonella* populations can be associated with interrelated parameters of litter pH, ammonia and moisture content (Santos et al. 2005) as well as water activity (Carr et al. 1994). These survival patterns indicate that within a litter environment a combination of parameters can be responsible for the continued presence of both these pathogens.

Thus an understanding of these interactions will aid in the development of simple management strategies which are based on such physical parameters aimed at pathogen reduction. An understanding of such parameters can aid in the development of strategies that can be adopted not only for varying litter re-use practices but for different bedding material as well.

Chicken litter - a source of *Salmonella* and *Campylobacter*

Used litter following a chicken cycle can be a source of residual *Campylobacter* and *Salmonella*, raising concerns of the possible transfer of these organisms through to the next chicken cycle (post treatment). The present study has demonstrated that prior to push-up both *Salmonella* (~10^3 MPN/g) and *Campylobacter* (~10^7 MPN/g) were present at varying stages of the chicken cycle. *Salmonella* was intermittently present while *Campylobacter* occurred at the latter stages of the chicken cycle. There is a close interrelationship between litter and faecal populations (Santos et al. 2005). These relationships are of significance in litter that has had a previous influence of chickens on it, such as re-used litter.

The litter re-use procedures vary across countries and there are universal concerns with regards to possible pathogen transfer across flocks. Multiple sampling of chicken litter during the growing period across three consecutive flocks monitored on four farms in Nova Scotia resulted in 16% of the used litter from two farms using wood shavings being positive for *Salmonella* (with 13 different serovars) (Long et al. 1980) with concerns of transfer across broiler cycles. Similarly the possible role of litter in the perpetuation and transmission of *Campylobacter* has been demonstrated. SPF chicks, placed on contaminated litter showed the intestinal shedding of *C. jejuni* within 5 days with that shedding persisting for 46 days (Montrose et al. 1985). Montrose et al. (1985) were concerned about these implications for broiler-producers, who generally utilize litter for five consecutive cycles, each of 49-55 days in duration in the USA. Such concerns can be addressed by creating an understanding of pathogen reduction mechanisms that may be possible between chicken cycles. The present study was designed to address such issues within an Australian context.

Litter treatment and piles

During the present trials litter was treated via a push-up process in which litter is piled within the shed as a windrow, a common method adopted in Queensland. This process is simple, with the natural changes occurring during this short push-up process resulting in the variation of key litter parameters such as pH, temperature, moisture content and water activity of the pile which influence pathogen die-off. Hence these parameters were all tested in the current trials. Poultry litter has been treated via pile-up in order to enable a composting process to contribute to pathogen die-off (Brodie et al. 2000). As well "in-house" composting has also been carried out to achieve the same outcome (Macklin et al. 2006). The present set of trials also demonstrated significant pathogen die-off, especially *Campylobacter*, followed by *Salmonella* and *E. coli* (indicator organism) during in-house pile treatment via a push-up process.

Whilst push-up after a cycle is not common in the USA, "stacking" of used litter is common when litter is destined as feed for cattle (Jeffrey et al. 2001, Bush, 2007 #3207). "Stacked litter" also undergoes changes in the piles such as pH, temperature and water activity (Jeffrey 2001). Such changes are monitored over a longer duration though the outcomes are a useful comparison to the current work. Essentially the stacking of litter in the USA is similar to the push-up of litter in an Australian context. In one study *Salmonella*, *Campylobacter*, *E. coli* 0157 were absent at the point of stacking and when tested 2 and 4 weeks later (Jeffrey et al. 1998). It is possible that the absence of the pathogens in the initial testing was a result of the age of that litter when initially stacked. In the present study the piles made essentially soon after pick-up and thus did not consist of aged litter. The Donnybrook litter had been through a single cycle with Laravale having a component of used litter (cycle 2 litter kept). The levels of pathogens in the litter before pick-up were ~10^3 MPN/g (*Salmonella*) and ~10^6 MPN/g (*Campylobacter*), with these organisms showing a die-off during push-up cycle.

Pathogen die-off in piles

In the present study *E. coli* the common indicator organism was not detected at Laravale in all pile locations tested at the end of the push-up cycle (days 4, 5 and 6 of the three litter piles). At Donnybrook *E. coli* was detected in one pile position at the end of the first pile study and in four of five pile positions in the second pile study (day 5). This may have been due to the lower temperatures prevailing in the Donnybrook pile (~ 45ºC) within some pile locations. However, both *Salmonella* and *Campylobacter* were not detected at the end of the pile-up process on both farms though showing a variable presence at the early stages. In the present study *Campylobacter* showed a marked die-off, around 6 log reduction within 12 -24 hours of the push-up based on the initial high levels of *Campylobacter* present at the latter stages of pick-up.

Research conducted by the Universities of Delaware, Auburn and Louisiana State suggests that the process of stock piling litter between flocks eliminates coliforms and *Salmonella* (Malone 2008). The process also reduces *Cl. perfringens*, total aerobic bacteria and total anaerobic bacteria by 50, 10-30, and 60-80 percent, respectively (Malone 2008). Studies (Jeffrey et al. 2001) that targeted the longer term stacking of piles have also looked at pathogen survival. Artificially inoculated stacks showed a maximum recovery of *E. coli* up to 32 hours (corresponding to a 5 log reduction of *E. coli*) while both *Salmonella* and *Campylobacter* were recovered up to 28 and 2 hours respectively. Thus there was a 4-6 log reduction in *Salmonella* and a 2-3 log reduction in *Campylobacter* within the above short period of time (Jeffrey et al. 2001).

In another instance both *E. coli* and *Salmonella* (introduced into mesh bags at around $10^{4.6}$ and placed in experimental piles of litter) were detected on day 1 but not on day 4 in either aerated or non-aerated piles. (Kwak et al. 2005). Kwak et al. (2005) suggested that conditions

other than the availability of air contributed to the short survival of both these organisms. *S.* Typhimurium inoculated into litter contained in dialysis bags and placed in different pile locations survived closer to the ground (temperature 37ºC) even though eliminated from rest of the sites which represented 99% of the study sites (Bush et al. 2007). It is possible that the survival at ground level reported by Bush et al. (2007) was possibly due to supportive conditions present at that location. Thus variable conditions within a pile may allow pockets of survival (or re-growth), a factor that needs to be taken into account when managing litter piles.

Clostridium perfringens and *Bacillus* spp., both spore forming organisms, were not impacted by the pile-up process in the current study. *Clostridium perfringens* is known to be commonly found within the chicken production system on dirty walls, dirty fans, fly strips, dirt outside of the house entrance as well as being known to proliferate in carcasses of dead birds left inside the house (Oviedo-Rondón 2008). An 80% reduction of *Clostridium perfringens* was observed during the composting of chicken manure with a moisture content of 40% being suggested as a key aspect of the composting process (Guillouais and Couronne 2003). While in the present study moisture levels of 40% were observed at times, the short time frame of the litter piles may explains the lack of impact of *Clostridium perfringens* as compared with the lengthy true composting process studied by Guillouais and Couronne (2003). Overall the conditions during pile-up do not seem to have the ability to target the elimination of spores.

Bacillus spp. are present in the broiler gastrointestinal tract (Barbosa et al. 2005) and are known to thrive in alkaliphilic environments (Yumoto 2002) as well as have the ability to produce antimicrobial substances (Stohl et al. 1999). Thus there is possibility that this group of organisms can also contribute to anti-pathogenic activity in litter, particularly as the study has shown that they continued to be present in re-used litter.

In the cycle 2 litter pile at Laravale there was evidence of the possibility of *Salmonella* re-growth, as has been observed in composting (Sidhu et al. 2001). In the present study *Salmonella* was not detected in the spread litter prior to pile-up but was detected at some pile sites during the pile-up process. It should be noted that this pile presented negative for *Salmonella* at the end of the pile-up process probably due the conditions prevailing at the time. However, the high levels of *Salmonella* at times during the pile life mean that *Salmonella* could have continued to be present if supportive conditions in the pile had prevailed right through the pile-up process.

Pathogens and the "dual litter environment" through the chicken cycle

Despite the short duration of the pile life this study has shown effective pathogen die-off during the push-up process. Many factors could be contributory and will be discussed later. Once spread at the grow-out end this treated litter can continue to undergo physical changes. However the current practice (new litter in the brooder end and old litter in the grow-out end) means there is a "dual litter environment" within the shed and perhaps "dual set of conditions" impacting on pathogen survival across a chicken cycle.

Once chickens were moved across there was no measurable differences in terms of the levels of *E. coli* in both the new shavings (brooder end) and the old litter (grow-out end) were found in the current study. Levels of *E. coli* in pine shavings, following chicken placement, of 10^7 to 10^8 cfu/g have been reported (Macklin et al. 2005), the level also found in the present study. In contrast treated litter supported ~ 10^4 cfu/g of *Clostridium perfringens* once spread while the new shavings had no detectable *Clostridium perfringens*. However shortly after chick placement the litter showed similar levels *Clostridium perfringens* within a week. At the latter stages of the chicken cycle there is not much difference between this "dual environments" across both farms for both *E. coli* and *Clostridium perfringens*.

In the case of *Salmonella* this "dual environment" does present some variation. There was a tendency for a higher level of this organism across both farms in the brooder end litter compared to the grow-out end. As well there was a higher prevalence and greater variety of serovars in litter originating from the brooder end compared to the grow-out end. *Campylobacter*, though present at high levels between weeks 5-6 in litter, after post pick-up there was a tendency to die off more in re-used litter than the new.

Temperature and pathogens in litter

Temperature has an impact on pathogen survival and true composting processes have been known to reach high temperatures of 58 – 69ºC (due to microbial activity) (Tiquia et al. 1996). The creation of windrows (and the addition of a carbon source) is a feature of full composting to enable increases in temperature targeting such pathogen inactivation (Cekmecelioglu et al. 2005).

During the present trial these simple in-shed piles (or windrows) did achieve temperature increases due to inherent microbial activity, especially at the surfaces which had more contact to oxygen than the cores. The cores did show gradual increases in temperature but in most instances not as high as some of the surface temperatures. These surface temperatures are thought to be higher because of the activity of the aerobes on the surface of the pile and the lack of penetration of air into the depths of the pile (Kwak et al. 2005). Reduced aerobic and anaerobic counts have been linked to internal temperatures around 55ºC lasting for 40 h in composted pine shavings (Macklin et al. 2006). In "stacked" litter, areas near the surface heated more rapidly than the deeper portions of the pile, where temperatures peaked between 2 -10 days of stacking and then declined (Jeffrey et al. 2001). This was similar to the pattern observed in the present set of trials where piles lasted for a maximum of 6 days.

Across the two study farms overall temperatures of around 60 - 65ºC were achieved mainly at the top of the pile. The roof also can be acting as insulation in this region of the pile. Such high temperatures combined with of a pile life of a maximum of 6 days could contribute to pathogen reduction.

However in the present trial, these high temperatures (60 - 65ºC) were not attained in all pile locations and duration of such temperatures was variable. In some instances temperatures were below 40ºC with uneven heating of the pile with cores heating very slowly. However pathogen die-off was demonstrated in all piles. The following published trials can provide some explanation. A single batch of litter was placed in shallow 1 L open containers (no heating) as well as being prepared as stacked piles (1.0 m long, 1.2 m deep, and 1.0 m wide) (heating); and both systems were inoculated with *Salmonella*. The *Salmonella* count declined drastically with time in the containers as compared to the piles, indicating that heat was not the sole contributor to pathogen die-off (Kwak et al. 2005). A similar observation was made when *S.* Typhimurium was introduced into the pile as well as in litter held at room temperature protected from light. Heating of the pile did occur with subsequent *Salmonella* die-off. However as there was only 30% survival in post stacking litter controls held at room temperature, the conclusion that heat alone was not responsible for *Salmonella* elimination under these conditions was drawn (Bush et al. 2007). Similarly even though variable temperatures were observed across pile locations in the present study pathogen die-off did occur by the end of the life of the pile (4-6 days), indicating a role for factors other than temperature.

Once treated (after push up) the spread litter at Laravale had temperatures of around 20 – 25ºC, whilst the spread litter at Donnybrook had higher temperatures around 26 - 33ºC, perhaps due to continuing microbial activity. This is supported by their respective water activities once spread (Laravale ~ 0.76 – 0. 80 and Donnybrook ~0.91 – 0.93). In terms of

Salmonella the Laravale litter had a water activity inhibitive of *Salmonella* growth while the Donnybrook litter had a level supportive of *Salmonella* with a potential risk at his stage.

pH and pathogens in litter

The waste generated by the chicken is in the form of uric acid which breaks down to ammonia (Carlile 1984). Ammonia can have a significant killing effect on pathogens in deep stacked litter (Kwak et al. 2005). In contrast to heat treatment reducing pathogens, this is a chemical treatment effect. Chemical studies carried out to stimulate these conditions (addition of 3% urea nitrogen to sludge) has shown an increased pH of 9.2 within 1 h and 9.5 within a few hours. These high pH levels contributed to a release of high concentration gaseous ammonia causing the main sanitation effect (Vinneras 2007). However while *Salmonella* was inactivated within 5 days the treatment was found not to have a significant effect on spore-forming *Clostridium* spp. (Vinneras 2007). During the present study *Clostridium perfringens* remained unaffected and *Salmonella* was eliminated following the push-up periods of 4-6 days. Gaseous ammonia is also released at pH levels of 7 and above (Pope and Cherry 2000). pH along with water activity has been linked with the death of *Salmonella* in poultry litter (Payne et al. 2007). The accumulation of free ammonia in poultry manure was also shown to be an important factor in inactivation of *S*. Typhimurium (Himathongkham *et al*. 2000). The pH levels in the piles at both Laravale and Donnybrook were slightly greater than 9.00 most of the time and thus the production of gaseous ammonia could have had a contributory role to pathogen die-off. However, a lack of correlation between the increases in litter pH and associated ammonia emission from turkey litter has also been demonstrated (Santos et al. 2005). This leads to a suggestion that other factors or a combination of factors can play a role in pathogen die-off mechanisms in litter in the addition to pH.

Water activity and pathogens in litter

The concept of water activity to manage spoilage of foods has been around for a long time (Troller 1972). It is not the water content (moisture content) but the water activity of a food system that governs microbial growth and toxin production (Tapia et al. 2007).

Several studies (Carr et al. 1994, Carr et al., 1995, Hayes, et al. 2000, Payne, et al. 2007) have evaluated the relationship of water activity and the presence of *Salmonella* in surface litter in poultry houses. For example the use of this parameter has provided useful data in terms of comparing the implications of *Salmonella* survival in sheds constructed with different floor types (wood, earthen or concrete) (Carr et al. 1994). Thus litter water activity appears to be a very versatile parameter for understanding the litter – *Salmonella* dynamics. Indeed for litter environments water activity could be used a "predictive tool" for *Salmonella* proliferation and if present as a "management tool – a concept explored during the current trial. Hence in the present study litter water activity was assessed both during the pile-up process and spread litter (with and without chickens).

Similar studies for *Campylobacter* and litter were not common.

There is a difference between the water activity of litter and the moisture content of litter. The moisture content refers to both the "free" (or actively available to the organism i.e. water activity) and the "bound" (or water of hydration/adsorbed water) in litter (Opara et al. 1992). This "Free water" (i.e. water activity) is directly related to microbial growth (rather than the bound water) and is associated with factors such as the ionic balance, the amino acid pool that is the available for the use of the organism and genes that manipulate water transport across the membrane of an organism (Labuza and Altunakar 2007). Water activity is also a measure of free molecular water or the equilibrium relative humidity (Carr et al. 1995) within the litter micro environment which has a direct impact on the growth potential of *Salmonella*.

Studies on broiler litter at the University of Maryland have shown the following to be a general indicator for the presence of *Salmonella* -. an aW of 0.75 – 0.83, 0.83 – 0.90 and 0.90 – 0.96 being negative, a transition zone and positive respectively (Carr et al. 1994). As well, a water activity of < 0.84 is regarded as effective for reducing *Salmonella* populations (Payne et al. 2007). The minimum water activity required for growth varies with the species for example *Salmonella* has a minimum range of 0.95 – 0.91, *Clostridium perfringens* 1.00 - 0.95 and *Campylobacter* 0.98 (Tapia et al. 2007, Taoukis and Richardson 2007). However, even if they cannot grow these organisms can survive low water activity and growth can be initiated given the correct conditions (Labuza and Altunakar 2007). Hayes et al. (2000) have suggested even though water activities that are supportive of *Salmonella* growth may occur thorough the poultry house some "hot spots" of contamination do occur with the rest of the areas having a potential to become contaminated. It has been suggested that holding chicken manure at a water activity of 0.89 can reduce *Salmonella* levels by a million fold and hence adjustment of suitable ventilation rates for a few hours to achieve appropriate water activity can help to control *Salmonella* in poultry houses (Himathongkham et al. 1999). Similarly a delay in colonisation of *Campylobacter* has been shown in litter under low relative humidity compared to high relative humidity in the shed environments (Line 2006).

A model developed for stacked poultry litter found the parameter with the greatest single influence on temperature (of the pile) was water activity followed by pH (Jeffrey et al. 2001). These workers concluded that high water activities and pH were a by-product of bacterial metabolism. Similarly the factor that had the most significant correlation to on-farm *Salmonella* status was water activity (Carr et al. 1995). Thus either directly or indirectly this parameter water activity does seem to have an influence on the ability of litter to support the survival of pathogens such as *Salmonella*.

Across all five litter push up cycles at both Laravale and Donnybrook the subsequently spread litter generally demonstrated water activities around 0.83 and moisture contents of 15% to 25%. There was a clear difference between the piled litter and the spread litter, with both being on the extreme end (but opposite ends) of the non-supportive ranges for *Salmonella.*

The piles at Laravale were associated with around 35% moisture content, perhaps due to washing of sheds at that time. Himathongkham *et al*. (1999) working on the effects of water activity of chicken faeces and *Salmonella* growth have suggested re-examining the practice of washing sheds, as it can result in high water activities (if piles were wet).

In the current study most pile locations exhibited water activities in excess of 1.00. Water activities in excess of 0.95 – 1.00 were generally not associated with *Salmonella* populations in litter (Payne et al. 2007, Santos, et al. 2005). There were some instances where *Salmonella* was present during these higher water activities in certain trials and in certain pile locations. Some of the possible explanations for these situations are a difference between global and local water activity around the particle, where the particle pore size has a role to play in the microscopic air-water distribution (local water activity) (Hayes et al. 2000) with this local water activity ultimately dictating survival. Nevertheless water activity seems to be a useful parameter in managing both piled litter and spread re-used litter prior to chicken placement.

Factors supporting pathogen re-growth

The pile environment is a dynamic and changing environment. It contains nutrients and subject to prevailing parameters such as temperature patterns pH, moisture and water activity pathogen re-growth is a possibility. However, re-growth is more associated with the latter stages of the composting process via the re-introduction of the pathogen by vectors. In the present situation the pile temperatures may have been a contributory factor, along with its short generation time of the pathogen. *S*. Typhimurium when tested on irradiated raw chicken meat demonstrated a generation time of 0.74 hours (44 minutes) (McKay et al. 1997). In

contrast *Campylobacter* when tested under laboratory conditions in liquid media demonstrated a generation time of 90 minutes (Rollins et al. 1983), though the organism has a very remote potential of growth due to other prevailing conditions in litter. Thus given the right conditions these organisms have the potential to replicate fairly rapidly in litter.

Salmonella contamination of surfaces and the possibility to contaminate treated litter

The presence of *Salmonella* within the shed environment may have an impact on the pile up process. This may explain why *Salmonella* was absent prior to push up in spread litter yet present in the piled litter as seen in one Laravale litter cycle. Bhatia *et al*. (1979) tested dust samples collected across the barn before the litter was spread as a possible source of contamination of feed, litter and flock. A relationship was demonstrated between the *Salmonella* serovars isolated from both dust and litter (Chinivasagam *et al*. 2008, Bhatia *et al*. 1979). A similar approach was adopted during the present trial. The presence of *Salmonella* on shed surfaces was tested through the cycle on shed surfaces such as drinkers, feeders, ropes, ledges, air vents, curtains, heaters and feeder pipes through the cycle. While *Salmonella* was absent from the surfaces tested the possibility of cross contamination should be taken into account during litter pile up operations.

Factors contributing to pathogen persistence or die-off in piles

While short duration does contribute to the die-off of *Salmonella*, *Campylobacter* and *E. coli* during the (maximum 6 days) all piles examined in this study achieved a die-of of both *Salmonella* and *Campylobacter*. Similarly, studies in the USA have shown that simple stacked poultry litter piles (not turned or aerated) did not support the survival of *Campylobacter*, *E. coli* or *Salmonella* for more than a few days (Jeffrey et al. 2001). While covering of the piles can result in higher pile temperatures both covered and uncovered piles achieved pathogen die-off (Macklin et al. 2006, Jeffrey, et al. 1998). As and example the study of Jeffrey et al. (1998) demonstrated that, when testing piles of poultry litter (intended for cattle feed) the internal temperatures exceeded 54.4ºC in covered piles and 40 – 46.3ºC in uncovered piles. However neither pile type yielded either *Salmonella* or *Campylobacter*.

A range of factors such as litter temperature, pH (related ammonia production) water activity (and related moisture content) as well as intrinsic flora in litter all have the potential to contribute to *Salmonella* and *Campylobacter* die-off in the pile environment. These parameters can act either singly or in combination effecting to achieve litter pathogen die-off or indeed pathogen re-growth and thus need to be managed.

Physical parameters to monitor pathogen reduction

Based on the findings of this study simple physical parameters such as litter temperature, pH and water activity could be used as a measure to understand or monitor pathogen die-off both in litter piles and spread litter.

However, as discussed these parameters seem to act in combination leading to the effect of a single parameter being further supported by another. For example while certain pile locations may not reach the key temperatures for inactivation a suitable pH can still result in high ammonia generation which will have a bacteriocidal effect.

Conclusions

Litter re-use (and subsequent management) varies from country to country and even among producers within a country. In South East Queensland litter treatment commonly occurs via a simple push-up process and the turn-around times are short due to the need to move on to the

next production cycle. The spread litter at the-grow out end following the pile up process was shown to have physical parameters that are regarded as non supportive of pathogen survival. This was shown by the absence of pathogens in the spread litter prior to the movement of chickens across to the grow-out end. All evidence from the present study confirms that the push-up process is a suitable means to eliminate the high levels of both *Salmonella* and *Campylobacter* (when present) prior to the next cycle. With appropriate management this can be achieved with a pile life of 4-6 days and thus relatively short turn-around times.

Appendix 1

Field observations for Donnybrook Farm, June –July 2007 (Chicken Cycle 3)

Date	Day of Cycle	Chickens set down/picked up	Dust	Additional Comments
6/6/07	5	40,000 in on 1/6/07		
11/6/07	10		Not much dust inside (none outside – no fans)	Chickens in half shed (on new litter side) Chicks very clean
26/6/07	25		Dust more apparent and easier to collect	Chickens noticeably larger
2/7/07	31			Litter very muddy in places, drinker line leaking Chickens seem packed in tightly Don't seem very active Noticeable aggressiveness between birds (due to lack of space)
9/7/07	38	15,540 removed on 6/7/07	Very dusty in shed	Same problems with litter being waterlogged as last time Majority of chickens seem inactive
16/7/07	45	2nd pickup of 3,150 on 13/7/07	Very dusty in shed	Drinker 1 – old litter wet (probe 5) Chickens fairly inactive
23/7/07	52		Slightly more dusty than last week Wind kicking up a lot of dust outside	A lot of feathers outside Chickens slow to react Logger – on reuse side, drinkers 1+3 were wet (probes 5+8) Chickens put on 300-500g since last week (Info from farm manager)
27/7/07	Post Background	21,310 out on 27/7/07		Started pickup at around 12.30AM A lot of feathers around outside Doors all open A lot of vehicles in the area kicking up dust.

Field observations for Laravale Farm, August - October 2007 (Chicken Cycle 6)

Date	Day of Cycle	Chickens set down/picked up	Dust	Litter	Additional Comments
28/8/07	0	32,390 (in on 3rd Sept 2007)	None.	Parts of litter not completely covering floor.	Shed Dimensions: Length of shed – 117m, Width of shed – 13m, Number of bays – 33, Curtain - Bay 15. Litter Pile Dimensions: Length – 10.5m, Width - 6.5m, Height – 2.16m, Sprayed inside with formaldehyde on 27/8/07. Curtain down.
10/9/07	7	None.	More dust than last week.	Litter sparse in places on new side.	Hotter than average (31deg C). Chickens grown well.
17/9/07	14	None.			None.
24/9/07	21	None.		Litter sparse in places on new side.	None.
1/10/07	28		A lot more dust apparent inside.		Chickens noticeably larger and well packed in (getting crowded). Cool pads on.
8/10/07	35	16, 500 out at 11.30 am			
15/10/07	42	2,000 today			Pickup started @ about 10 am.
22/10/07	49	Remaining birds 13, 890 will be picked up on 26/10/07			There is a side door at bay 17 on right side as you look from fan end.

Chapter 3

Background information for the establishment of a code of practice for "in-shed pathogen reduction" of chicken litter (to be finalised in collaboration with industry)

The information in this section forms the basis for a consultation process with industry to develop a suitable code of practice for piled litter. The code of practice could be based on some of the easy to measure parameters such as temperature, pH and water activity.

Table 2.1 describes the dimensions of the pile examined in this study. It is possible that much smaller piles could have very different results. However, for the dimensions typical in this study, height 2 m, length 7 – 28 m, depth 3.5 – 7.3 m, the current study suggests broadly similar physical and microbiological parameters will be found.

This leads to the conclusion that if a pile (of roughly the same dimensions) undergoes a push-up process in shed the changes occurring within piles will not show great variation. These changes will be both physical and microbial. All these changes that occur ultimately have an impact on the die-off of the two key pathogens tested, *Salmonella* and *Campylobacter*. *E. coli* an indicator organism was present through one entire pile cycle in one instance only. However die-off did occur in all other trials. Thus *E. coli* could be used as a simple organism of treatment efficacy.

In summary, the following litter parameters have collectively contributed to pathogen die-off in piled litter

- temperatures achieved (and their durations) at different pile locations
- pH levels achieved and their duration at different pile locations
- water activity levels achieved and duration at different pile locations
- microbial activity based on suitable growth conditions prevailing within the pile

A summary of key physical parameters

Temperature

Table 3.1 provides an overall summary of the key temperatures reached in all five trials.

Whilst it is acknowledged that the simple pile up processes that occur within the shed are not typical composting processes, some guidance in terms of a typical temperature that can be deemed sufficient for "pathogen kill" can be sought from guidelines that have been developed for human waste – an area where a range of international guidelines are available. The USEPA guidelines for composting of biosolids are one such comprehensive guideline (USEPA 1993).

As can be seen in from Table 3.1 the top, middle and bottom surfaces have generally reached reached temperatures in an excess of 55ºC, a temperature deemed as suitable for inactivation of pathogens in sewage sludge (USEPA 1993). These USEPA guidelines stipulate a temperature of 55ºC for 3 consecutive days for within vessel composting or the static aerated pile composting method, whereas a longer duration (15 days with turning) is suggested for windrow composting (USEPA 1993).

Table 3.1 Summary of temperature changes occurring in pile locations

Farm	Pile Location	Temperature Cycle 1	Temperature Cycle 2	Temperature Cycle 3
Donnybrook	Top 100 mm	58-60 (24 hrs)	54-55 (16 hrs)	
	Top 200 mm	64-65 (24 hrs)	63-64 (38 hrs)	
Laravale	Top 100 mm	64-65 (75 hrs)	56-59 (18 hrs)	62-65 (44 hrs)
	Top 200 mm	64-65 (75 hrs)	66-68 (23 hrs)	59-62 (32 hrs)
Donnybrook	Middle 100 mm	60-62 (27 hrs)	45-48 (15 hrs)	
	Middle 200 mm	63-64 (32 hrs)	66-67 (23 hrs)	
Laravale	Middle 100 mm	63-64 (25 hrs)	60-63 (24hrs)	58-60 (16 hrs)
	Middle 200 mm	63-65 (42 hrs)	66-68 (72 hrs)	63-65 (50 hrs)
Donnybrook	Bottom 100 mm	56-57 (35 hrs)	41-43 (17 hrs)	
	Bottom 200 mm	58-59 (31 hrs)	47-48 (44 hrs)	
Laravale	Bottom 100 mm	60-62 (45 hrs)	62-63 (20 hrs)	47-49 (18 hrs)
	Bottom 200 mm	63-64 (39 hrs)	35-38 (10 hrs)	45-47 (29 hrs)
Donnybrook	Core 1 M	64-65 (31 hrs)		
	Core 1.5 M		66-69 (22 hrs)	
	Core 2 M	58-59 (36 hrs)	43-44 (26hrs)	
Laravale	Core 1 M	65-66 (48 hrs)	49-51 (14 hrs)	54-55 (39 hrs)
	Core 1.5 M			
	Core 2.0 M	48-49 (46 hrs)	48-50 (15 hrs)	46-47 (95 hrs)

Table 3.2 Summary of pH, water activity and moisture content occurring in all pile locations

Farm	Pile Location	pH Cycle 1	pH Cycle 2	pH Cycle 3
Donnybrook	Top	8.74	8.73	Not done
	Bottom	8.78	8.67	
	Core	8.38	8.57	

Farm	Pile Location	pH Cycle 1	pH Cycle 2	pH Cycle 3
Laravale	Top	9.08	8.98	9.10
	Bottom	8.93	9.02	9.08
	Core	8.55	8.78	8.81

Farm	Pile Location	aW Cycle 1	aW Cycle 2	aW Cycle 3
Donnybrook	Top	1.02	1.02	Not done
	Bottom	1.02	0.99	
	Core	0.98	1.07	

Farm	Pile Location	aW Cycle 1	aW Cycle 2	aW Cycle 3
Laravale	Top	1.01	1.07	1.05
	Bottom	1.02	1.06	0.99
	Core	0.97	1.06	1.00

Farm	Pile Location	Moisture Cycle 1	Moisture Cycle 2	Moisture Cycle 3
Donnybrook	Top	29.83	27.3	Not done
	Bottom	31.65	22.4	
	Core	34.60	27.8	

Farm	Pile Location	Moisture Cycle 1	Moisture Cycle 2	Moisture Cycle 3
Laravale	Top	30.73	33.45	32.87
	Bottom	34.55	33.25	33.78
	Core	36.27	38.88	29.40

In-shed piling does have a possibility of an insulation capacity. The piles are protected, enabling fairly high temperature ranges to be achieved. Thus, based on the above data, an overall temperature of 55ºC for duration of 4-6 days (based on the present trials) seems like a suitable benchmark for effective "pathogen kill'. Of course this key parameter in the case of chicken litter is well supported by pH and water activity. Both of these factors along with temperature can be acting in unison to achieve "pathogen kills" especially where temperatures have failed to reach the optimum recommended temperature of around 55ºC. Work carried out in the USA on in-house chicken litter Windrowing/Composting has set the goal of achieving 130ºF (54.4ºC) or greater within the first two days and to maintain these windrow temperatures for a minimum of three to five days. This USA work also acknowledges the contribution of both ammonia as well as competing organisms to pathogen die-off in chicken litter (Malone 2008). Higher temperatures can contribute to elimination of beneficial microorganism associated with the composting process.

Water activity, moisture and pH

Table 3.2 provides an overall summary of the levels of pH, water activity and moisture content seen in the various piles in this study. Water activities in excess of 1.00 were achieved at most pile locations. Similarly, a pH of around 8.5 was recorded at most pile locations (supportive of the release of ammonia) was also recorded in litter originating from different pile locations. These physical parameters contribute to "pathogen kill" during the pile-up process.

Additional factors

Additional in-shed practices are also vital so that cross contamination does not interfere with a successful pile-up process.

Some of these factors are as follows:

- The required windrows (one or two) should be formed shortly after chicken removal
- Any large litter clumps that are present need to be broken up so that a compact pile devoid of air pockets (to aid a uniform heating process) can be created
- If washing of shed does occur during the push-up process, especially the latter stages of push-up, the pile should not be wet.
- Once the pile-up is completed the litter needs to be spread over clean surfaces

Simple monitoring equipment for a quality management process

Development of an in shed quality management process for piled and spread litter (prior to chicken placement) is a possibility. The development of such process may require simple in shed monitoring parameters documented. The following are some such equipment that can generate data in cost effective and simple manner.

- A water activity meter for testing both pile (and surface litter) water activities.
- Small temperature loggers can be scattered across the pile (especially at the surfaces), where the temperature data could be down loaded later

A possible QA system

Whilst subject to industry consultation the beginnings of a possible QA process are set out below:

- The required windrows (one or two) should be formed shortly after chicken removal – windrows should be no more than 2 m in height and with a minimum width of around 4 m
- Any large litter clumps that are present need to be broken up so that a compact pile devoid of air pockets (to aid a uniform heating process) can be created
- If washing of shed does occur during the push-up process, especially the latter stages of push-up, the pile should not be wet.
- Monitor temperature of the pile, A temperature of 55^{o}C for a minimum of 3 days is the goal
- Maintain the pile for a minimum of 5 days
- Once the pile-up is completed the litter needs to be spread over clean surfaces
- Water activity of the spread litter (if moist) will be an indication of a possible risk for *Salmonella* contamination and thus an optional monitoring tool

In the event of future regulatory requirements for microbiological monitoring the current study indicates that the most effective monitoring would be for *E. coli* on the final day of pile life in a composite sample representative of the bottom surface area of the pile.

Based on the outcomes of the current study a *E. coli* level of < 100 cfu/g could be easily achieved

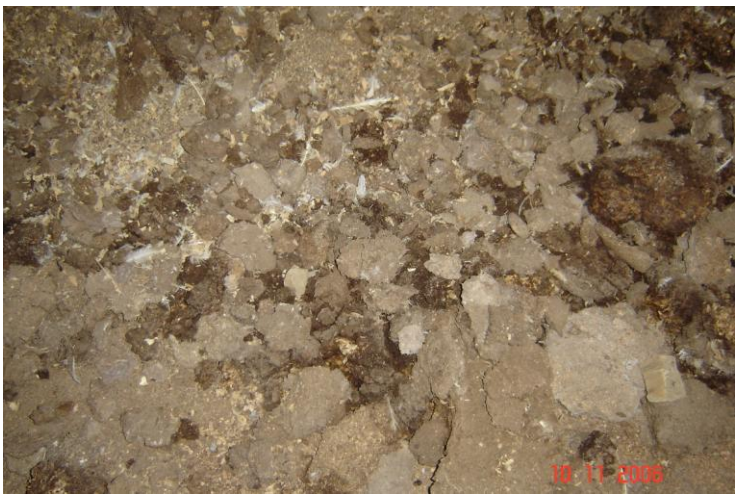
Illustrations relevant to a possible QA system

On the following pages are photographs situations relevant to the potential QA system.

Figure 3.1 The windrows should be formed shortly after chicken removal –and should be no more than 2 m in height and with a minimum width of around 4 m. The surrounding floor should be clean and dry. The pile should be compact and with no large clumps of litter

Figure 3.2 During the shed cleaning process the pile should not be wet. The litter from the pile should be spread on a dry clean surface

Figure 3.3 If possible large clumps of litter should be broken down during the windrowing process



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Chapter 4

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