



AUSTRALIAN POULTRY CRC

FINAL REPORT

Optimising methods for multiple batch litter use by broilers

Program 3

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PROJECT LEADER: Professor Steve Walkden-Brown

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methods for multiple batch litter
use by broilers**

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Project No. 06-15

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1 Introduction

1.1 Background to the project

The Australian broiler industry has a well-recognised problem with both the supply of bedding material in some regions, and with the disposal of spent litter. The main litter materials used are shavings, sawdust, chopped straw or rice hulls and prices now range from \$20-\$30/m³ when available. In 2001 it was estimated that the annual requirement for bedding material was 1.17 million m³ costing \$10.78 million with the industry producing 1.6 million tons of spent litter (Runge et al., 2007). In 2008 Australia produced 463 million broilers (ABARE, 2009), a 22% increase on 2001 production (379 million) so the 2001 litter volumes can be expected to have increased commensurately to 1.43 million m³ of fresh litter and 1.95 millions tons of spent litter by 2008.

The increasing volume of spent litter has become more difficult to dispose of, primarily due to food safety concerns with direct application in the horticulture industry, but also due to environmental concerns about nutrient accumulation and leaching into waterways. The typical levels of N and P in Australian spent litter are 2.4–4.2% and 1.6–2.4% respectively (Runge et al., 2007). The industry is quite rightly investigating alternative sources of bedding material and improved treatments and value adding of spent litter. This project is focussed on the level of usage of litter material within the industry and an examination of the feasibility of reducing litter material use and disposal by re-using it for chicken production. The potential consequences of litter reuse for chickens are large, with each reuse almost halving the requirement for new materials and the amount of spent litter to be disposed. It could be that the best way to add value to spent litter is to run another batch of birds on it following an appropriate treatment during the cleanout period.

The starting hypothesis for this project is that reuse of litter by successive batches of broiler chickens will be necessary in the future, and that it is feasible provided the correct methods of managing and treating litter are used. The major goal of the project will be to develop such methods.

The broiler industry produces 1.2 to 1.5 tons of waste products in the form of poultry litter per 1000 broiler chickens when reared in a single-batch litter (Coufal *et al.*, 2006). In Australia 85% of chickens are reared on fresh litter materials following complete shed cleanout between batches (East, 2007). The remainder are reared on previously used litter using a variety of systems. Systems may involve total reuse of litter or partial reuse. In the former case, litter is usually heaped and partially composted at the end of the batch, without addition of moisture, in heaps approx 2 m high, 6 m long and 3 m wide for a week or so before re-spreading. Significant temperatures are achieved in such heaps for considerable periods (>56 °C at 23 cm into the heap) although their effectiveness in reducing pathogen loads has been questioned (Runge et al., 2007). In the case of partial litter reuse, the brooder area is typically cleaned out and disinfected fully with the old litter transferred to the growout section where it may be partially composted together with the material from that area. The brooder area is then cleaned thoroughly and given completely new bedding. Other variations include placing a thin layer of new material (2.5–5.0 cm) in the brooder section following partial composting and re-spreading. In some cases the old litter is not disturbed beyond removal of caked litter. Full cleanout and a new batch of litter may be triggered by completion of a fixed number of cycles of reuse, or a disease outbreak or breach in biosecurity.

Currently there is marked variation between companies in the extent to which litter is reused for multiple batches. Multiple litter use is more widely used in some major poultry producing countries such as the USA, and other methods of litter treatment are widely used in that country. These are aimed mainly at reducing ammonia production and include incorporation of acidifying agents such as aluminium sulphate (alum), organic acids, antimicrobial compounds to target urease producing organisms (eg paraformaldehyde flakes) or hygroscopic materials to sequester moisture in the litter.

The main reasons for the high rate of fresh litter usage in Australia are concerns about chicken health, biosecurity and performance. The principal concerns are with carryover of poultry pathogens in the litter, particularly viruses (Groves, 2002), and increased ammonia concentrations particularly in winter

when heating is being used and ventilation rates are lower. There are also concerns about the carryover or accumulation of zoonotic bacteria but this issue is being dealt with in a separate project. This project addresses the former two issues.

This project was initially submitted as a PRP in the 2005 round of applications with the title “Environmental and health consequences of litter reuse in broiler farms”. It was modified considerably in subsequent discussions with industry and funding agencies and the final research proposal was approved in 2007 for 2.5 years funding, July 2007 to December 2009. Project progress was overseen by an industry steering committee comprising:

Program Leader (Ian Farran) – Chair

Dr Margaret Mackenzie – Inghams Enterprises

Dr Jorge Ruiz – Baiada Select Poultry

Dr Liam Morrisroe – Bartter Enterprises

Mrs Peta Easey – Cordina Chicken Farms

Mr Gary Sansom – Grower representative

The investigators in the project were

Prof. Steve Walkden-Brown, UNE (Project Leader)

Dr Fakhru Islam, UNE (Project Scientist)

Dr Ben Wells, Wells Avian Consultancy

Mr Mark Dunlop, Qld. Department of Employment, Economic Development and Innovation

Dr Peter Groves, Zootechny Pty Ltd

1.2 Project objectives

The project was driven by the broiler industry’s twin problems of difficulty in accessing sufficient litter material, and difficulties in disposing of it after use. reuse of litter by chickens is one approach to reducing these problems. The overall hypothesis under test is that litter can be safely reused by successive batches of chickens without loss of productivity or chicken wellbeing provided it is done properly.

The overall objective of the project was to:

- Determine survival times of key viral poultry pathogens in litter under a variety of litter management practices; and
- Develop specific methods of litter treatment and management to enable safe reuse of litter by broilers under typical Australian conditions

The project proposed to fulfil these objectives in a phased way by doing the following:

Comprehensively reviewing existing information on the subject including a risk assessment on the pathogens and practices most likely to threaten chicken health, welfare and performance (See Appendix 1. Literature review and Risk assessment);

Developing and validating novel methods for measuring viral pathogen load in litter (Chapter 2);

Conduct research to devise optimum methods for litter reuse taking into account the geographical, breed, nutrition and litter type variation that exists within the Australian chicken meat industry (Chapters 3-6). This work will specifically involve:

- Testing the effects of a range of litter partial composting treatments on the temperature, pH and chemical composition of litter on commercial farms;
- Testing the effect of these same treatments on survival/infectivity of viral pathogens and coccidia in the litter; and
- Investigation into the temporal and spatial distribution of ammonia in sheds, and the effects of litter reuse on ammonia production.

1.3 Project methodology

Methodology for each specific experiment is detailed in the experimental chapters. Particularly for Chapter 2, this includes new method development. An overview of project locations and methodology is provided below.

1.3.1 Overview of project locations and research team

The project had several geographically disparate components, as summarized below.

University of New England (Fakhrul Islam and Steve Walkden-Brown)

- All isolator experiments (Chapters 2, 3 and 4).
- Generation of deliberately infective litter for experiments in Chapters 2, 3 and 4.
- Serology for experiments in Chapters 3 and 4. For Chapter 2 it was done at Birling Avian Laboratories.
- qPCR for experiments in Chapters 2 and 3.
- Litter analysis for experiments in Chapters 3 and 4.
- Setting up, calibration and testing of environmental monitoring equipment (ammonia, dust, temperature, air speed).
- Literature review.
- Data analysis and write up.

Cordina Chicken Farms (Sydney) (Ben Wells, Peta Easey, Mark Johnstone)

- Provision of field litter for experiments in Chapters 2 and 3.
- Location of litter treatment experiment in Chapter 3 (a single farm).
- Location of ammonia & dust distribution experiment in Chapter 4 (8 farms).
- One of two companies for field monitoring of ammonia on new and used litter (Chapter 6).

Inghams Enterprises (Brisbane) (Margaret Mackenzie, Brett Richter, Kelly McTavish)

- Provision of field litter for experiments in Chapters 2 and 4.
- Location of litter treatment experiment in Chapter 4 (a single farm).
- One of two companies for field monitoring of ammonia on new and used litter (Chapter 6).

DEEDI QLD (Mark Dunlop)

- Advice on environmental monitoring equipment and calibration.

- Physical involvement in environmental monitoring in experiment in Chapter 3 (Sydney), days 1 and 2.

Bartter Enterprises (Liam Morrisroe)

Provision of field litter for experiments in Chapter 2.

1.3.2 Generation of infective litter at UNE

To test for positive litter transmission of pathogens, positive controls were used in Chapters 2–4. In each case a group of approximately 40 commercial broiler chickens was obtained at day old, placed on clean litter in a controlled environment room in the UNE animal house, and vaccinated or challenged with live virus (or coccidia) of strains under test. The nature and timing of these vaccinations varied and is described in each chapter. Birds were then kept until 28–35 days of age, allowing time for active shedding of virus onto the litter. The litter was then collected and used in experiments to test transmission of pathogens to chicks in the chick bioassay.

1.3.3 Chick bioassay to test litter infectivity (UNE)

The development and validation of this test is fully described in Chapter 2 and in the paper arising from this work (Islam et al., 2009). Briefly, groups of 10–11 SPF chickens (SPAFAS, Australia) were placed in positive pressure isolators and exposed to approximately 9 litres of chicken litter in two plastic “kitty litter” trays (Figure 1-1). Litter was also spread on the scratch paper. Chickens tended to actively explore in the litter as soon as it was introduced and many would sleep on it. Litter remained in the trays for approximately 3 weeks in decreasing amounts. At day 35 post exposure to litter, chickens were bled and sera examined for antibodies indicating exposure to infective virus. Treatments were replicated in two isolators generally giving 20 animals per treatment. Other measurements were sometimes made at this point or at a common age near this point as well.



Figure 1-1: Chick bioassay to test litter infectivity in positive pressure isolators. SPF chicks soon after exposure to litter (*left*) and close to day 35 post exposure to litter (*right*).

1.3.4 UNE isolator facilities

Main isolator facility.

The chick bioassay experiments in chapters 2–4 were mostly conducted in the main 24-isolator facility at UNE. The isolators are housed in a biological PC2 laboratory under constant negative pressure and

with all outgoing air HEPA filtered. Each isolator has a length of 2.05 m, width of 0.67 m and height of 0.86 m with a stainless steel frame. The floor is 2.5 mm stainless steel (304 2b) with 12.7 mm holes punched out with centres 17.45 mm apart staggered providing 49% open area. Isolators are positive-pressure soft-bodied with disposable plastic linings, gauntlets and gloves, disposed of after every experiment. Isolators are provided with temperature-controlled HEPA-filtered air via a central air supply system and air is scavenged from each isolator via a series of scavenger ducts and HEPA filtered on exit. Both inlet and outlet air supplies are under manual control via a variable speed controller, giving complete control over air-flow and isolator pressures. Isolators are individually fitted with heat lamps under separate thermostatic control, automatic waterers and feeders. The entire feed supply for each experiment is loaded into a large feed hopper for each isolator and sealed for the duration of the experiment. Temperature in each isolator is monitored constantly via a data logger and displayed on a computer screen in the facility. The entire facility has automated power backup via a 13 KVA generator. At the time of writing, nine major experiments have taken place in the facility without breakdown of biosecurity or other major problems. Photographs of the facility are included in Figure 1-2 and Figure 1-3.



Figure 1-2: Interior of isolator facility at UNE showing 24 isolators and main air inlet duct. This carries HEPA filtered, heated air to each isolator. Note the green feed hopper above each isolator.



Figure 1-3: Exterior of the isolator facility at UNE showing the plant room on the right and the main isolator facility in the middle with the air extraction and filtration system next to the people.

Other isolators. When the number of isolators required exceeded 24, additional isolators were deployed in climate-controlled rooms in the UNE animal house. These older style isolators were fixed body isolators with individual motors and HEPA filtered air intakes. Exit air was bag filtered back into the room. Heating was on a whole room basis. These isolators, ten of which are available at UNE, are illustrated in Figure 1-4 to Figure 1-6. When these isolators were used, treatments were generally able to be blocked to take account of location/isolator type.

1.3.5 Field litter treatments and transportation of litter to UNE

Field litter treatments comprised end of batch litter (Chapters 2–4) or litter that had been heaped, with or without turning at day 3 (Chapter 3), or litter that had been windrowed along the centre of the shed with or without turning at day 4 (Chapters 3 and 4). Litter was collected from a large number of sites to ensure a representative sample (see specific chapters for details, Figure 1-7 to Figure 1-9), mixed thoroughly, allowed to cool at room temperature, and then bagged in approximately 5 litre amounts in breathable bags (cotton fabric or reusable non-woven polypropylene green shopping bag) (Figure 1-10). These were then packed into a 60 L esky with bagged ice placed around the bags (Figure 1-11) and transported to UNE by overnight courier. This treatment had little effect on litter infectivity as shown in transport simulations with positive control litters in Chapters 2–6.



Figure 1-4: Older style fixed body isolators used in some experiments.



Figure 1-5: Chicks a day or so after placement in a fixed body isolator. Watering arrangement.



Figure 1-6: Chicks a day or so after placement in a fixed body isolator. Feeding arrangement.



Figure 1-7: Sampling day 0 litter at different depths.



Figure 1-8: Sampling litter in a windrow with a depth marker.



Figure 1-9: Sampling litter from a windrow at 25 cm depth.



Figure 1-10: Bags of litter prepared for transport.



Figure 1-11: Bags of litter placed in a 60 L esky with bagged ice for overnight transport to UNE.

1.3.6 Serological analysis (Birling, UNE)

Serology for Chapter 2 was carried out at Birling Avian Laboratories. Subsequently it was carried out at the UNE poultry health laboratory using commercial kits from IDEXX and TropBio and following the manufacturer's instructions. Samples were analysed in duplicate and positive and negative controls were included on each plate. Sample dilution and OD interpretation was based on the manufacturer's guidelines. Details are provided in Chapters 2–4. At UNE a new ELISA for MDV was developed based on the method described by Zelnik et al. (2004). The antigen was prepared by from vaccinal MDV (Rispens CVI 988). Full validation and evaluation of the assay has not been completed at this point, but more than 1000 samples from a wide variety of experiments has been tested and the assay has proven to be very sensitive and specific for MDV although it is not serotype-specific. Sensitivity at this stage appears to be greater than for qPCR detection of MDV in spleen. A full standard curve was included on each plate. Results from this assay are included in this report, but should be interpreted as indicative only at this stage. In this report, no threshold titre is set for negative values. Negative values are those for which no colour reaction was detected in the assay and all detectable colour reactions were recorded as positive.

1.3.7 Litter temperature measurements

Litter temperature was monitored continuously (15 min–1hour intervals) using either Tinytag climate data loggers (Gemini Data Loggers, Chichester, UK) (Chapter 2) or iButton[®] DS1921 data loggers (Evolution Education Ltd, Bath, UK). For the field experiments in Chapters 3 and 3, 80–100 iButton data loggers were deployed across the various treatments and depths to provide accurate estimates of mean temperature.

1.3.8 Litter analysis (UNE)

Litter pH measurement.

pH was determined with a pH meter. Two and a half grams of litter samples were weighed in a screw cap plastic jar, and 50 ml of Milli-Q water was added. The contents were shaken vigorously for one minute to mix thoroughly. The mixture was kept at room temperature for 30 minutes with vigorous shaking every 5 minutes. The pH meter was calibrated using standard buffers of pH 4 and 7 before reading the sample. The electrode was thoroughly rinsed in between sample reading. The instrument was calibrated with pH 7 buffer after every 4 samples.

Dry matter measurement.

Litter dry matter was determined by weight difference after drying approximately 50 g of litter at 80 °C for 48 hours.

Sample preparation for chemical analysis.

Dried litter samples were ground with a fine grinder then sieved using 0.2 mm and 0.05 mm sieves separately. The 0.2 mm sieved products were used for determining potassium (K), sodium (Na), phosphorus (P) and other trace elements using an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES, Carlo Erba Instruments, Strada Rivoltana, Milan, Italy) and the 0.05 mm products were used to determine carbon (C) and nitrogen (N) content using a solid sample analyser (Carlo Erba NA 1500).

Determination of K, Na, and P and trace elements.

This was done using an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES): This machine is capable of measuring total elemental analysis on particulate free liquid samples from 175 nm to 785 nm. Before analysis, the samples were subjected to sealed chamber digestion using perchloric acid (HClO₄) and hydrogen peroxide (H₂O₂) following a method described previously (Anderson and Henderson, 1986). In brief, approximately 0.2 g of litter material was weighed (keeping the record of the exact weight of the material) in a 50 ml borosilicate reagent bottle. Two ml of a mixture of a 7:3 (v/v) of HClO₄ and H₂O₂ and allowed to digest overnight at room temperature. The bottles were sealed tightly and placed into a warming oven at 80 °C for 30 minutes. After cooling, a further 2 ml of H₂O₂ was added and incubated at 80 °C for one hour. A final volume of 25 ml was made by adding deionised water. The mixture at this stage contained 3.9% (0.65N) HClO₄. The mixture was filtered through a 0.45 µm glass fibre filter before analysis through ICP-OES using full wavelength coverage for micro and macro molecules.

Determination of N.

This was done using a Carlo Erba NA 1500 Solid Sample Analyzer: 200 mg of each sample was weighed into a 5×8 mm tin cup and loaded into the autosampler of the analyser. The sample was then introduced into the combustion furnace, which was maintained at 1030 °C. The sample and container melted, where tin acted as a catalyst promoting a violent reaction (flash combustion) in a temporarily oxygen enriched atmosphere. The combustion products were carried by a constant stream of ultra high purity helium through a reduction column at 500 °C and then through a filter of 2:1 magnesium perchlorate:quartz turnings, which adsorbed the water from combustion. The elemental nitrogen and carbon in the helium stream enter the chromatographic column (80 °C) for separation according to molecular weight and flow through to the Tracermass. The component beams arrived at collectors and their positive charge produced an electric current, which was amplified and passed to the data system. Both nitrogen and carbon were recorded on individual channels of the source controller and results given as % N, atom %, and % C, delta. The whole procedure was operated by the automated process of the Carlo Erbo Analyser.

1.3.9 Air measurement of ammonia, dust temperature and wind speed

Aerial ammonia concentrations in ppm were measured using VRAE7800 Hand Held Gas Surveyors (Geotech Environmental Equipment, Inc., Colorado, USA) calibrated against a 50 ppm NH₃ standard sample (Figure 5-2). This meter has a detection range of 0–50 ppm for NH₃.

Particulate matter in the shed air was measured (mg/m³ air) in using DustTrak™ Model 8520 aerosol monitor (TSI Inc, Minnesota, USA). The machine measures dust particles ranging from 0.1–10 µm

Introduction: Objectives and Methods

using light scattering technology with a laser photometer. These measurements include the **respirable** dust fraction comprising particles with a diameter less than or equal to 5 μm , which penetrate into the gas exchange region of the lungs, and is therefore the most hazardous particulate size. It also includes part of the total **inhalable** dust fraction, which is the fraction of airborne particles which enters the nose and mouth during normal breathing and is made up of particles with diameter less than or equal to 100 μm .

Wind speed, humidity and temperature were recorded with a Kestrel Weather Meter K4000 (Nielsen-Kellerman, Inc., PA, USA).

All equipment had a data logging function and were caged in a custom made frame at the required heights while in the shed (See Chapter 5, Figure 5-3).

2 Development and optimization of a bioassay to determine viral load in poultry litter

Experiments 1 & 2 — LT07-C-CB1 and CB2

Start: 11/09/07

Completion: 19/11/07

AEC: UNE AEC07/118

AEC: UNE AEC07/119

2.1 Introduction

The aim of this project was to support attempts to increase the level of litter reuse in the broiler industry by developing methods for measuring the infectivity of litter and using these to optimise litter reuse practices. The reluctance to reuse litter can be based upon concerns about the survival in litter of viral pathogens (Groves, 2002) and the difficulty in detecting and enumerating infective viruses in the environment relative to bacteria. Molecular tests for viral presence suffer from the limitation that they may not be measuring viable virus or infectivity as they cannot differentiate infective from non-infective pathogens.

Our response to these problems was to develop a chick bioassay of litter infectivity as a key part of the project. In brief, groups of susceptible disease-free chickens were exposed to infective litter for a fixed period of time, and then grown out to 35 days of age in isolators. Detection of the pathogens of interest will be confirmed by either the pathology induced during this period or serological or molecular diagnosis at the end of the 35-day period. The assay as developed will be qualitative or semi-quantitative rather than fully quantitative.

This chapter summarises our findings on the evaluation and validation of a chick bioassay.

It reports the findings of two experiments carried out at UNE.

- Experiment 1—LT07-C-CB1 (see section 2.2)—aimed to produce fresh infective litter for use in development of the bioassay and for use in a transportation simulation to test the effects of transportation to UNE on litter infectivity.
- Experiment 2—LT07-C-CB2 (see section 2.3)—was our first attempt at developing the bioassay, using SPF and commercial broiler chickens of two ages to determine the infectivity of infective litters prepared at UNE or obtained from poultry companies.

These experiments and their findings are detailed below. They have also been published (Islam et al., 2009).

2.2 Experiment LT07-C-CB1: Production of infective litter for developing a litter bioassay of viral infectivity and testing the effects of simulated transportation.

(Commenced: 11 Sept 2007, Completed: 08 Oct 2007)

2.2.1 Introduction

The main objectives of this experiment were:

- To produce litter known to be contaminated with the vaccinal strains of IBV, IBDV, NDV, and CAV. This litter was eventually used as a positive control for the development of the chick bioassay in the main experiment.
- To test whether transportation of litter material (simulated) reduce the viability of viruses.

2.2.2 Materials and methods

A total of 40 chickens (Cobb broiler) were raised in floor pens (4.5 m²) in a climate-controlled room in the UNE Animal House (chickens were received from a 49 weeks old parent flock at Lynwood 1). Approx 8 cm of pine wood shavings was laid on the floor. Chickens were fed broiler starter ad lib for the first 14 days then changed to broiler finisher. Normal brooding temperatures were used; starting at 35 °C on day zero and declining by 1 °C every 2 days until a temperature of 21 °C was reached.

Vaccination was performed as described in the Table 2-1. At day 28 of age, all chickens were removed and litter was used in the subsequent experiment.

Table 2-1: Vaccine and vaccination schedule of the experiment.

Vaccine	Source	Strain	Dose	Batch No	Age of chickens at inoculation	Route of inoculation
Vaxsafe IBD	Bioproperties	Strain V877	>102.4 EID50/dose	BN IBD06271B	14 days	Eye drop
Vaxsafe ND	Bioproperties	Strain ND V4	>106.0 EID50/dose	BN NDV050350C	14 days	Eye drop
Vaxsafe IB	Bioproperties	IBV Ingham's strain	103.0 EID50/dose	BN IBI 061582A	21 days	Eye drop
Steggles CAV vaccine	Intervet/Neil Sammons	Strain 3711	101.575 CID50/dose		21 days	Oral, x10 dose used

To confirm successful infection/vaccination, on day 28 of the experiment (various days post vaccination) serum samples were collected from 12 individual chickens and sent to Birling Avian Lab for serological assay for ND (HI assay), IBD (ELISA), IB (ELISA) and CAV (ELISA).

Transport simulation (*UNE-Tran* litter). On day 28 of the experiment, 32 litres of litter from the group was collected, placed in 4 x 8 L muslin bags and placed into a 60 L esky together with 2 x 2 L frozen water containers to simulate a transportation of field litter samples to UNE from the field (*UNE-Tran*). The esky was placed in a controlled climate room for 24 hr with the temperature set to cycle from 14–35 °C. Temperature was monitored continuously inside and outside the esky using TinyTag[®] data loggers (Figure 2-1).

The following day (day 29), another 32 litres of fresh litter was collected and placed directly into isolators as part of experiment 2 (*UNE-Dir*).

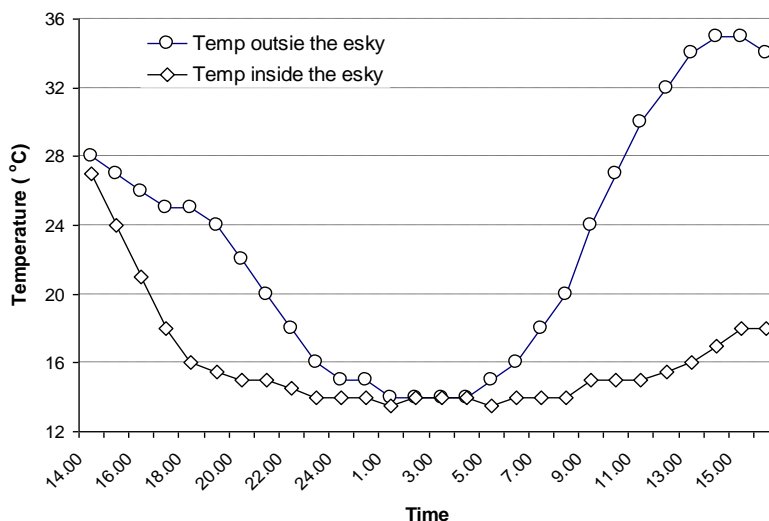


Figure 2-1: Temperature profile of the *UNE-Tran* litter that was kept in an esky with four litres of frozen water and placed in temperature control chamber at simulated warm transportation temperatures in a diurnal rhythm. Temperatures inside and outside the esky were recorded using Tinytag® data loggers.

2.2.3 Results and Discussion

Serological analysis revealed following results:

- 12/12 (100%) samples were positive for NDV (HI assay) (Serum sample collected two weeks following vaccination)
- 1/12 (8.3%) samples was positive for CAV (ELISA) (Serum sample collected 7 days following vaccination)
- 11/12 (91.7%) samples were positive for IBD (ELISA) (Serum sample collected two weeks following vaccination)
- 4/12 (33.3%) samples were positive for IBV (ELISA) (Serum sample collected 7 days following vaccination)

Vaccination was successful for all viruses. Vaccinal strains of ND (V4) and IBD (Inghams) established a high rate of infection and seroconverted almost all the vaccinated chickens. However, IBV (strain Ingham) induced seroconversion in only one third of the chicken population and the Steggle's Strain 3711 CAV vaccine caused seroconversion in less than 10% of birds. This poor seroconversion rate was not surprising as the serum samples were collected only at 7 days post vaccination.

The transportation simulation revealed that despite high external temperatures, temperatures inside the esky were maintained below 20 °C throughout once they had cooled. Heat is therefore unlikely to be a major inactivator of viruses in transported litter.

2.3 Experiment LT07-C-CB2: Development of a bioassay for infectivity of viral pathogens in poultry litter. Effects of chicken type and age, source of litter and transportation status.

(Date commenced: 08 Oct 2007, Date completed: 19 Nov 2007)

2.3.1 Introduction

The overall aim of this experiment was to begin development of a chick bioassay for litter infectivity based on continuous exposure of chickens to infective litter in isolators, with pathological and serological end points as markers of infectivity.

The specific objectives of the study were:

- To detect the presence of infective viral pathogens in poultry litter from a variety of sources.
- To determine whether unvaccinated commercial broiler or SPF chickens are best to use for the assay. Commercial broiler chickens may contain maternal antibody (mab) to many of the pathogens under test whereas the SPF chickens do not.
- To determine whether exposure to litter from day old, or 1 week old of age is preferable.
- To develop and test a method of effective transportation of litter from industry to the testing site at UNE.
- To determine the effect of such transportation on litter infectivity.
- To determine the optimum end point of detection for each virus involved.

The experiment was designed to test the following hypotheses:

1. SPF chickens will provide a more sensitive bioassay of infection than mab+ broiler chickens.
2. SPF chickens will have a higher mortality rate than broiler chickens
3. Chicks exposed to litter at day old will have higher mortality than those exposed at 7 days of age (particularly SPF).
4. Litter will be able to be transported without appreciable loss of infectivity.
5. Serology of chicks at 35 days post exposure will provide an adequate measure of exposure to infective IBV, IBDV, NDV, and CAV.

2.3.2 Materials and methods

The experiment utilized a $5 \times 2 \times 2$ factorial design with two replicates plus two uninfected control treatments. The factors and levels in the experimental design are:

- Five litter sources (*UNE-Dir*, *UNE-Tran*, Field Litters (*FL1*, *FL 2* and *FL 3*);
note: *UNE-Dir* is the litter produced in the earlier experiment LT07-C-CB1 used directly and *UNE-Tran* is the same litter used following a transfer simulation protocol (described in section 2.2.2 on page 11).
- Two chicken types (SPF and Cobb commercial broiler female); and
- Two initial exposure ages to the litter (days 1 and 7 of age). Chickens of the two ages were kept together in the same isolator with toe web marking to identify age.

In addition there was one control isolator for each type of chicken without exposure to any litter material (negative control).

The experiment utilised 22 soft-bodied, positive pressure isolators in the UNE isolator facility; 20 for the main factorial experiment and 2 for the negative controls. On day -7 ten day old chickens of either SPF white leghorns or Cobb broilers were placed in their respective isolators (7 day exposure group). On day zero, a further 10 day-old chickens of each type were added to the chickens placed a week before (Day 1 exposure group) providing a total of 20 chickens per isolator and 440 chickens in total. The two batches of chickens were permanently marked by toe-web cutting and placed in the same isolator.

On day zero (15 Oct 2007), 32 L of litter materials were collected from the various company or contract farms, using a detailed sampling protocol to ensure representative samples were collected. Samples were placed in 4 x 8 L cloth bags, placed in 60 L eskies with 4 L of frozen water and transported to UNE via overnight courier. All three litter samples from participating poultry companies arrived on the following day (day 1, 16 Oct 2007). Chickens were exposed to the litter material on the same day. Each isolator had 8 L of litter placed in it using two plastic kitty litter trays. Thus each litter sample was used in 4 isolators (2 SPF chickens and 2 Cobb broiler chickens). The litter in these trays remained in the isolators until it was completely depleted, several weeks later. The two control isolators had no litter material or trays placed in them.

Condition of litter upon arrival at UNE. Three field litters from three different poultry companies were used in the experiment. The companies were Cordina Chicken Farms, Inghams Enterprises and Bartters Enterprises. The litters were coded as *FL 1*, *FL 2* and *FL 3* (not in order). The condition of the litters upon arrival at UNE and prior to placement into the isolators were as follows:

- *FL 1*: Litter temperature was 13.1 °C. Dry, friable litter.
- *FL 2*: Ice was completely melted and some water had leaked leading to partial (mild) litter wetting. The temperature recorded was 17 °C.
- *FL 3*: Litter was collected from a broiler flock aged 52 days. The litter material was saw dust, reused following partial composting. The flock was vaccinated against IB, ND and MD (HVT). On opening the temperature was 16.9 °C. The litter was dry, dark and friable. The ice bottles had melted completely but the litter was reasonably cold.
- *UNE-Tran*: The litter was light and friable, containing significantly less faecal material than the farm litters. The temperature on opening was 18 °C.

All mortalities were examined for gross pathology on post-mortem. At day 28 of the experiment (day 21 post-exposure to litter), four SPF chickens from each isolator (two from each age group) were humanely killed and blood samples were collected and serum separated (these samples are not analysed yet). On day 42 of the experiment (day 35 post-exposure to litter), blood samples were collected from the survivors, serum separated and stored. Chickens were then humanely killed and weighed. Thymic atrophy was scored (0–3, 3 most severe), and the bursa and spleen weighed for SPF chickens only. Spleen samples (from individual chickens) were also collected at the termination of the experiment.

Serum samples were then analysed for serology for IB (ELISA), CAV (ELISA), IBD (ELISA) and ND (HI) at Birling Avian Labs. DNA was extracted from the spleen samples for MDV assay. Serum samples were assayed for FAV8 by ELIA (TropBio).

2.3.3 Statistical analysis

Mortality patterns were analysed by survival analysis using the product-limit (Kaplan-Meier) method. The log rank and Wilcoxon statistics were used to test for homogeneity between the groups. Mortality data were (coded as 0 for dead and 1 for alive) also analysed by analysis of variance (AOV) using generalised linear model of binomial data. Live weight and organ weight data were analysed using AOV with a mean separation by Tukey's HSD test and least square means (LSM) are presented. Difference in the proportion of chickens with positive serology for the different diseases was analysed by either Chi-square analysis or Fischer's exact test. Serology positive–negative data were also

analysed using AOV (GLM) of binomial data. All analyses were carried out using JMP version 6 (SAS Institute Inc., NC, USA).

2.3.4 Results and Discussion

2.3.4.1 Survival

Survival analysis revealed that there was a significant effect of chicken type ($P>0.002$) but not initial exposure age ($P=0.16$) on the survival rate of chickens. There was a higher survival rate in the SPF (96%) than broilers chickens (88%). Further analysis showed that there was a significantly lower survival rate for broiler chickens exposed at day 7 compared to day 1 and either of the SPF chicken groups (Figure 2-2).

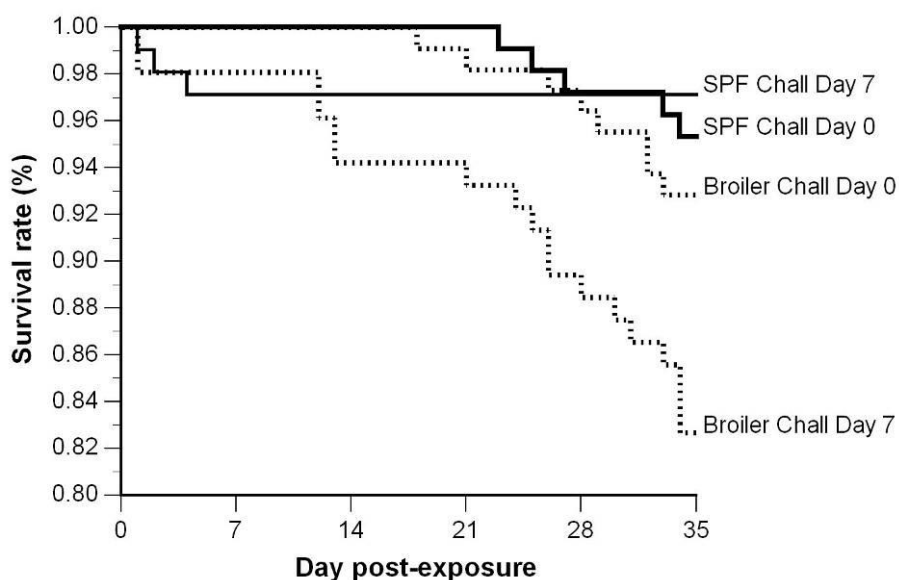


Figure 2-2: Survival curve of chickens from day 1 to 35 following exposure to the litter materials. The age of chickens exposed at day 7 is 7 days greater than for the groups exposed on day 1. Control chickens were also included in the analysis.

Analysis of variance of mortality data revealed that there was a significant effect of initial exposure age ($P>0.02$), but not chicken type ($P=0.12$) and litter source ($P=0.17$) on the mortality of chickens. There was also a significant interaction between the effects of chicken type and initial exposure age ($P>0.02$) and between the effects of litter source and initial exposure age ($P>0.07$) but not between the effects of chicken type and litter source ($P=0.16$). Overall, there was higher mortality in the group of chickens initially exposed on day 7 (12.6%) compared to the group exposed on day 1 (6.4%). Significantly higher mortality was observed in the broiler chickens initially exposed on day 7 than any other group (Table 2-2). When analysed for the effect of litter source, no significant difference in mortality was observed among the litter sources including control chickens. Causes of mortality were only determined by gross pathology on post-mortem and are presented in Table 2-3.

Table 2-2: Mortality of chickens throughout the experiment (all chickens included). The mortality of day 1 exposed group is from day 1 to 35 and for the day 7 exposed group is from day 0 to 42.

Chicken type	Litter Exposed Day 1			Litter Exposed day 7		
	Dead	Alive	Mortality	Dead	Alive	Mortality
Broiler	8	104	7% (5)	23	85	21% (16)
SPF	6	101	6% (9)	4	102	4% (6)

Table 2-3: Causes of mortality or euthanized during the experiment before the termination of the experiment.

Causes	Day 1 Exposed		Day 7 Exposed	
	Broiler (8)	SPF (6)	Broiler (23)	SPF (4)
Non-starter	2		6	1
Ascites	2		5	
Peritonitis	1		1	
Pericarditis			2	
Leg problem	1		1	
MD/Hepatomegaly			1	
Accidental			1	
NVL/Other	2	6	6	3

2.3.4.2 Live weight

Live weights of broiler and SPF layer type chickens were analysed separately since broiler chickens were single sexed females, whereas SPF chickens were mixed sex White Leghorns.

For SPF chickens, there was a significant effect of initial exposure age, sex and litter source ($P > 0.001$) on the live weight (LW) with no significant interaction between the main effects. Not surprisingly, the chickens exposed on day 7 had a higher LW (397 ± 6.0 g, age 42 days) than the chickens exposed on day 1 (335 ± 6.1 g, age 35 days) since they were 7 days older. The LW was higher in male than female chickens (male 392 ± 6.0 g vs female 340 ± 6.0 g). The effect of litter source was due to lower live weight recorded in chickens exposed to *FL 2* (333 ± 5.0 g) and *FL 3* (295 ± 5.0 g) litters compared to controls (366 ± 5.0 g), moreover, the LW of chickens on *FL 3* litter was significantly lower than that of chickens on all other litter groups except *FL 2* (Figure 2-3).

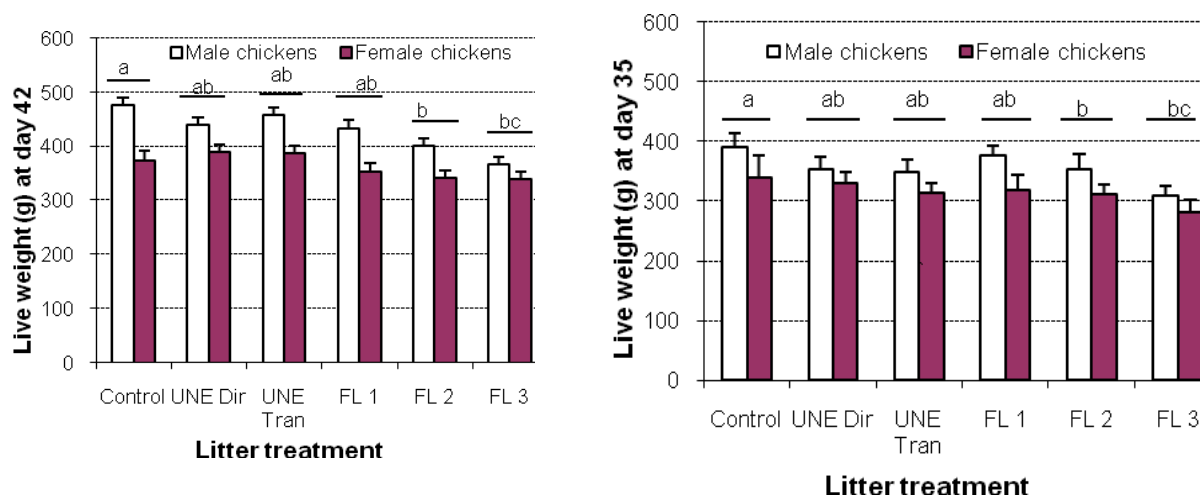


Figure 2-3: Live weight (LSM±SEM) of SPF chickens for various treatment groups. Treatments not having a common letter are significantly different ($P < 0.05$)

In broiler chickens, there was a significant effect of initial exposure age, litter source ($P > 0.001$) and their interaction ($P = 0.003$) on LW. Not surprisingly LW was higher in chickens exposed at day 7 (1851 ± 36 g, age 42 days) than day 1 (1475 ± 29 g, age 35 days). The LW of control chickens (1841 ± 25 g) and the chickens exposed to *UNE-Tran* (1750 ± 25 g) and *FL 2* (1740 ± 25 g) were higher than the LW of the other three treatments. It is interesting to note that the LW of broiler chickens reared on *UNE-Dir* (1498 ± 46 g) litter treatment was significantly lower than the chickens reared on *UNE-Tran* (1750 ± 52 g) litter. This suggests that there might be some pathogens (not under investigation) inactivated during the transportation simulation of the *UNE-Tran* litter. The interaction between initial exposure age and litter source was significant because the effect of litter treatments on LW was more pronounced in day 1 exposed chickens than those exposed to litter at day 7 (Figure 2-4).

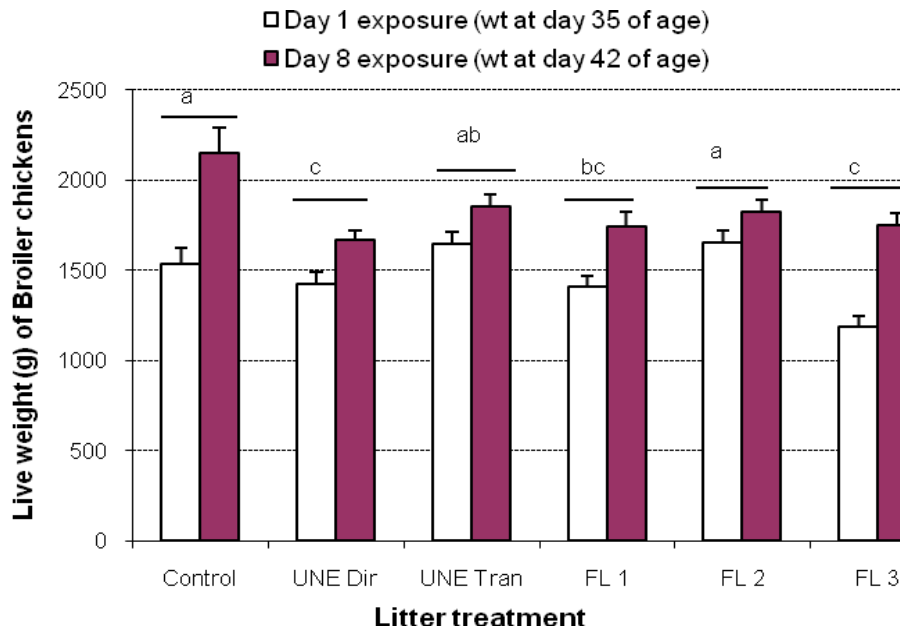


Figure 2-4: Live weight (LSM±SEM) of female broiler chickens for various treatment groups. Treatments not having a common letter are significantly different ($P < 0.05$).

2.3.4.3 Relative bursal weight

There was a significant effect of litter source ($P > 0.0001$) but not initial exposure age ($P > 0.10$) or sex ($P > 0.08$) on the relative bursal weight and there was no significant interaction between any of the main effects. The relative bursal weight was significantly lower in the chickens exposed to *UNE-Dir* and *UNE-Tran* treatments than any other litter-exposed groups (Figure 2-5).

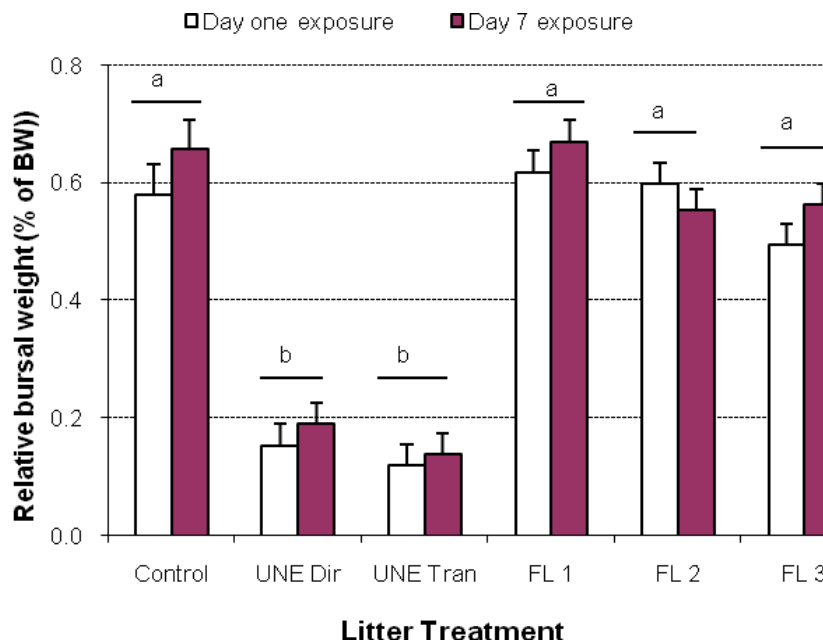


Figure 2-5: Relative bursal weight (LSM±SEM) of SPF chickens for various treatment groups. Treatments not having a common letter are significantly different ($P < 0.05$).

2.3.4.4 Relative splenic weight

There were significant effects of initial exposure age, sex ($P>0.001$) and litter source ($P>0.01$) on the relative spleen weight, however there was no significant interaction between any of the main effects. The relative spleen weight was higher in female (1.18 ± 0.023 g) than male (1.05 ± 0.023 g) chickens. The relative spleen weight was also higher in day 1 (1.19 ± 0.02 g) exposed chickens than day 7 exposed (1.04 ± 0.02 g) chickens. The effect of litter was due to higher relative spleen weight in the chickens exposed to *FL 2* and *FL 3* litters than chickens exposed to any other litters (Figure 2-6).

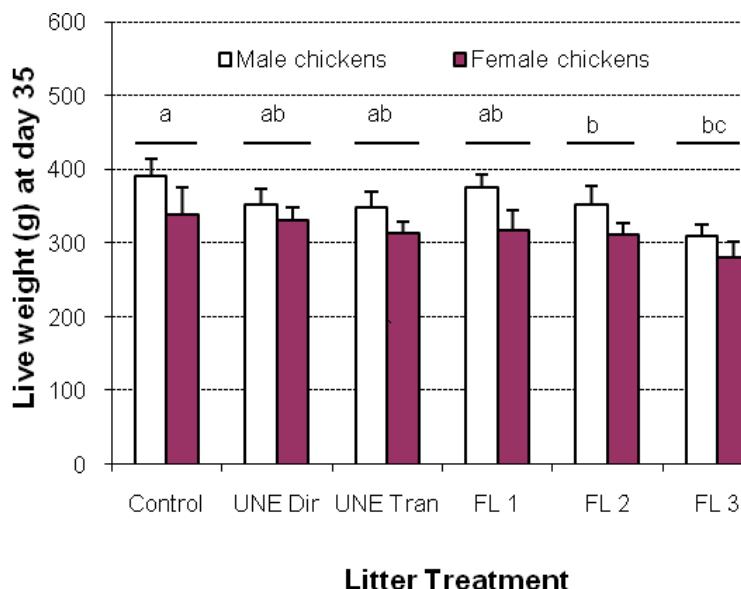


Figure 2-6: Relative spleen weight (LSM±SEM) of SPF chickens for various treatment groups. Treatments not having a common letter are significantly different ($P<0.05$).

2.3.4.5 Serology results

Chicken anaemia virus (CAV) and fowl adenovirus (FAV) were the most prevalent organism in litter samples from the field; it was detected from all participating farms. Infectious bursal disease (IBDV) and infectious bronchitis (IBV) were detected in one field litter sample only. No ND was detected in any of the participating farm. Overall serological assays detected a higher proportion of positives in SPF chickens than commercial broiler chickens.

Chicken infectious anaemia virus (CAV)

The litter infectivity bioassay detected CAV successfully in all participating field litters including both UNE litters. Field strains of CAV were transmitted through litter to both SPF and broiler chickens. Vaccinal CAV (Steggles strain 3711) also transmitted through litter to both types of chickens (Table 2-4)

Table 2-4: Serological results for CAV in the various treatment groups

Litter Treatment	SPF Chickens			Cobb Broiler Chickens		
	CAV+ (n)	CAV- (n)	CAV%	CAV+ (n)	CAV- (n)	CAV%
Control	0	14	0%	0	13	0%
<i>FL 1</i>	19	6	76%	7	29	19%
<i>FL 2</i>	27	2	93%	5	32	14%
<i>FL 3</i>	26	5	84%	21	16	57%
<i>UNE-Dir</i>	28	1	97%	6	30	17%
<i>UNE-Tran</i>	28	2	93%	2	28	7%
Total	128	30	81%	41	148	22%

Fowl adenovirus/inclusion body hepatitis (FAV/IBH)

Fowl adenovirus 8 was positive for all three field litters but not in UNE litters or in controls (Table 2-5). The serum samples from broiler chickens were not assayed for FAV titre. The titre of FAV antigen was also very high (Figure 2-7).

Table 2-5: Serological results for FAV/IBH in the various treatment groups

Litter Treatment	SPF Chickens			Cobb Broiler Chickens		
	FAV+ (n)	FAV- (n)	FAV%	FAV+ (n)	FAV- (n)	FAV%
Control	0	14	0%	0	10	0%
FL 1	13	11	54%	18	20	90%
FL 2	16	13	55%	10	20	50%
FL 3	30	0	100%	19	19	100%
UNE-Dir	0	27	0%	3	25	13%
UNE-Tran	0	28	0%	2	21	10%
Total	59	79	43%	52	105	33%

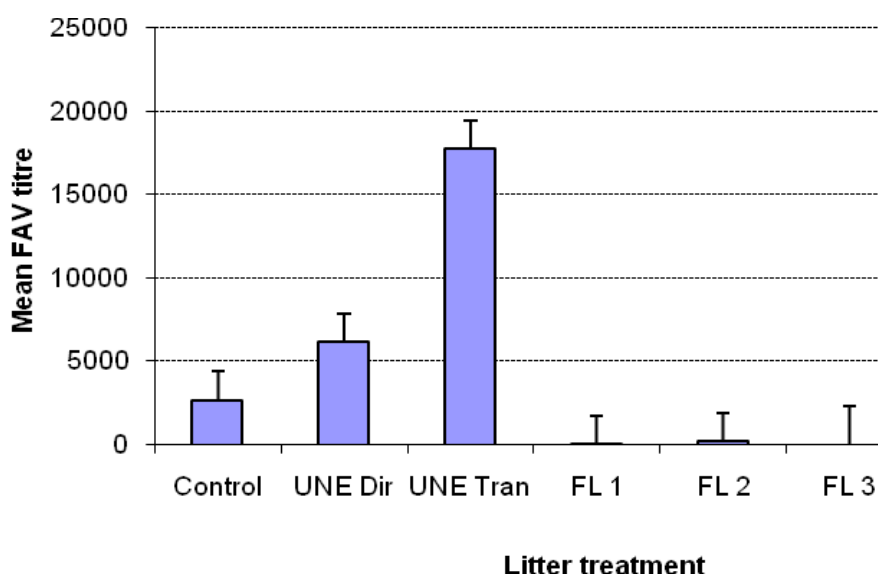


Figure 2-7: FAV/IBH titres (LSM±SEM) in the various treatment groups. Note: A titre less than 512 is considered as negative.

Infectious bursal disease virus (IBDV)

Field IBDV strains were picked up by both SPF and broiler chickens from FL 2 litter samples although the number of positive chickens was very low (Table 2-6). Vaccinal IBD strain (V877A) was transferable through litter in SPF chickens only but not in broilers, indicating the importance of maternal antibody.

Table 2-6: Serological results for IBDV in the various treatment groups

Litter Treatment	SPF Chickens			Cobb Broiler Chickens		
	IBDV+ (n)	IBDV- (n)	IBDV%	IBDV+ (n)	IBDV- (n)	IBDV%
Control	0	14	0%	0	13	0%
<i>FL 1</i>	0	25	0%	0	36	0%
<i>FL 2</i>	2	27	7%	1	36	3%
<i>FL 3</i>	0	31	0%	0	37	0%
<i>UNE-Dir</i>	27	2	93%	0	36	0%
<i>UNE-Tran</i>	30	0	100%	0	30	0%
Total	59	99	37%	1	188	0.5%

Infectious bronchitis virus (IBV)

The UNE litter was unable to transmit vaccinal strain of IBV (Ingham's strain) to either SPF or broiler chickens, although a significant number (25%) of donor chickens were positive for IBV antibody. Only two broiler chickens were positive for IB from the field litter groups and it was not repeated in SPF chickens (Table 2-7). Therefore the IBV transmissibility result was not conclusive.

Table 2-7: Serological results for IBV in the various treatment groups

Litter Treatment	SPF Chickens			Cobb Broiler Chickens		
	IBV+ (n)	IBV- (n)	IBV%	IBV+ (n)	IBV- (n)	IBV%
Control	0	14	0%	0	13	0%
<i>FL 1</i>	0	25	0%	0	36	0%
<i>FL 2</i>	0	29	0%	2	35	5%
<i>FL 3</i>	0	31	0%	0	37	0%
<i>UNE-Dir</i>	0	29	0%	0	36	0%
<i>UNE-Tran</i>	0	30	0%	0	30	0%
Total	0	158	0%	2	187	1%

Newcastle disease virus (NDV)

No NDV was detected in any of the litter samples (Table 2-8). While this may reflect a lack of NDV in field litters, the UNE litters also failed to transmit vaccinal ND strain (V4) to chickens although 100% of the tested shedder chickens sero-converted for NDV. Perhaps this strain of NDV is not readily transmissible through litter. Checking with Birling Avian labs confirmed that the positive controls used in the HI test worked as expected so it was not an assay failure.

Table 2-8: Serological results for NDV in the various treatment groups

Litter Treatment	SPF Chickens			Cobb Broiler Chickens		
	NDV+ (n)	NDV- (n)	NDV%	NDV+ (n)	NDV- (n)	NDV%
Control	0	14	0%	0	13	0%
<i>FL 1</i>	0	25	0%	0	36	0%
<i>FL 2</i>	0	29	0%	0	35	0%
<i>FL 3</i>	0	31	0%	0	37	0%
<i>UNE-Dir</i>	0	29	0%	0	36	0%
<i>UNE-Tran</i>	0	30	0%	0	30	0%
Total	0	158	0%	0	187	0%

Marek's disease virus (MDV)

Five spleen samples from each litter type (SPF chickens only) were assayed for MDV using real-time PCR. All the samples were negative for MDV.

2.3.4.6 Effect of chicken type

Significant numbers of both types of chicken were serologically positive only for CAV. Chi-square analysis revealed that, in general, a significantly higher proportion of SPF chickens (81%) were positive for CAV than broiler chickens (22%) ($P < 0.001$). This was also true of the vaccinal CAV used in the UNE litter treatments where more than 90% SPF chickens were positive for CAV with both litters whereas only 7–17% broiler chickens were positive (Table 1.4). Vaccinal IBDV was only detectable in SPF but not in broiler chickens. Clearly both CAV and IBDV bioassays were more sensitive in SPF than commercial broiler chickens.

2.3.4.7 Effect of initial exposure age

Chi-square analysis showed that there was no significant effect of the age of initial exposure on the proportion of chickens positive for CAV, IBDV and FAV in SPF chickens (Table 2-9). However, a higher proportion of broiler chickens was positive for CAV when exposed to litter at day 7 than day 1 ($P > 0.01$). This almost certainly is due to the decline in maternal antibody against CAV by day 7. This was reflected in the mortality data too (Figure 2-2).

Table 2-9: Serological results of CAV and IBD by initial exposure age (days)

Chicken type	Exposure day	CAV+	CAV-	CAV%	IBD+	IBD-	IBD%	FAV+	FAV-	FAV%
SPF	0	62	15	81%	28	51	35%	31	45	41%
	7	66	15	82%	31	48	39%	30	48	39%
Broiler	0	13	91	13%	1	103	1%	Not assayed		
	7	28	57	33%	0	85	0%			

2.3.4.8 Effect of litter transportation

Two vaccinal viruses (IBDV and CAV) transmitted effectively through UNE litters. Fisher's exact test revealed no significant difference in the proportions of chickens positive for IBDV between *UNE-Dir* (93%) and *UNE-Tran* (100%) litter groups ($P = 0.24$). Similarly there was no significant difference in the proportion of chickens positive for CAV between *UNE-Dir* (97%) and *UNE-Tran* (93%) ($P = 0.99$). These two results suggest that transportation simulation of UNE litter (*UNE-Tran*) did not affect the transmissibility of these viruses. However, significant reduction in LW of broiler chickens reared in *UNE-Dir* may be due to some other reason that is not revealed in the bioassays.

2.3.5 Summary and conclusions

The study demonstrated that:

- The proposed chicken bioassay proved very effective at detecting infective CAV and IBDV in litter, but not IBV or NDV, although it is not certain that the inability of detecting IBV and NDV was due to the absence of these viruses in the litter or due to poor transmissibility through litter.
- Simulated transportation of litter had no significant effect on the infectivity of litter as detected by the serological results of the bioassay.

- As expected, bioassays in SPF chickens were more sensitive than commercial chickens. Mortality of SPF chickens was not higher than broiler chickens and there appeared to be no serious ethical issues associated with their use, at least for the litter range tested. The results suggest that SPF chickens would be the animal of choice to use in the bioassay.
- As there was no advantage (in terms of sensitivity) or disadvantage (eg. early mortality) of exposing SPF chickens at day 1 or 7. From a practical point of view, it is preferable to expose the chickens at day 1 or 0 to shorten the length of the bioassay. If broilers or commercial off-sex layer cockerels were used, it would be preferable to expose the chickens at a later age after maternal antibody has waned.
- The three field litter samples used in the study varied slightly in pathogen load; this appeared to be reflected in BW, particularly in SPF chickens. The *FL 3* litter appeared to have the highest CAV and FAV loads while the *FL 2* litter had a low level of IBDV detected. Birds from these two treatments had the worst LW performance, particularly in SPFs, and some bursal atrophy. Interestingly the severe bursal atrophy induced by CAV in the UNE treatments did not penalise performance to the same extent—probably because of the limited number of pathogens in the litter of these treatments.
- Overall the experiments provided support for proof of concept for the bioassay of litter infectivity. The assay offers good prospects for being a useful tool to measure the efficacy of different litter treatments in reducing viral load in litter.

3 Effect of in-shed partial composting of litter on the inactivation of viral infectivity

Experiment LT08-C-CB3 & CB4

Start: 09/10/08

Completion: 15/12/08

AEC: UNE AEC08/133

3.1 Introduction

Reuse of litter by broiler chickens can reduce the environmental impact and cost of chicken production but uptake of the practice is limited by risks of pathogen carryover. Unlike some other major poultry producing countries, only a small proportion (~15%) of Australian broiler farms reuse litter in the shed (East, 2007). The reluctance to reuse litter in the Australian broiler industry is primarily based upon concerns on the animal health and productivity issues (Groves, 2003) particularly due to carry-over infection with pathogens, particularly viruses.

A small proportion of Australian broiler growers do reuse litter in the shed following partial composting by heaping or windrowing for 4–10 days, where a temperature of ~60 °C can be reached inside the litter heap or windrow. The aim of this study was to assess the effectiveness of such litter treatments over time on the inactivation of selected poultry pathogens under field situations.

Unlike bacteria, viruses are not easy to detect and quantify from poultry litter, as viruses do not grow in non-living media. Therefore, little or no data are available on viral survival rate in poultry litter following different litter treatments. As part of the current project, we developed and validated a bioassay to detect and quantify viral pathogens from poultry litter (Islam et al., 2009; Chapter 2) and this tool was used to measure change in litter infectivity following treatment in the present study.

The main objectives of this experiment were to:

Determine the efficacy of partial composting (heap and windrow) in reducing litter pathogen load.

Determine the decay profile of litter infectivity in partially composted litter (heap and windrow).

Determine the effect of litter composting (windrow vs heaping with or without turning) on the decay of viral infectivity.

Determine changes in temperature, pH and moisture over nine days in variously treated litter.

Determine changes in litter C, N, P and K over nine days in sheds containing litter processed through windrow or heap with or without turning.

The specific hypotheses under test were:

Infectivity of litter for viruses and coccidia will be reduced following heaping or windrowing.

Infectivity will be reduced more in treatments generating greater temperatures.

Heaping will be more effective than windrowing.

Turning will lead to increased inactivation.

Most of the benefits will be seen by day three.

Ammonia measurements during litter treatment will fall within acceptable levels (<25 ppm).

Aspects of this study are reported by Islam et al. (2010a).

3.2 Materials and methods

There were three major parts of the experiment, litter treatment experiment in the field, generation of positive control litter at UNE and the actual bioassay in isolators at UNE. The litter treatment experiment was conducted at a broiler farm in Sydney (Cordina Chicken Farms).

The broiler farm was situated in the Austral region, a highly dense chicken farming area. The farm had six conventional broiler sheds; each has a capacity of rearing 25,000 to 30,000 broiler chickens. The experiment was conducted in three sheds, Shed A, Shed B and Shed 4 (Figure 3-1). The description of the sheds is provided in Table 3-1.



Figure 3-1: Aerial view of the broiler farm with sheds identified by name.

There were three litter treatments: windrow (Win), heap turned (HT) with mechanical turning of the heap on day three, and heap without turn (H). Each shed was roughly divided into two to make six experimental units. The treatments were applied to the litter of half of each shed either on the day of complete removal of chickens or a day after. The above three treatments were applied in each half of the shed as shown in the Figure 3-2 to Figure 3-6.

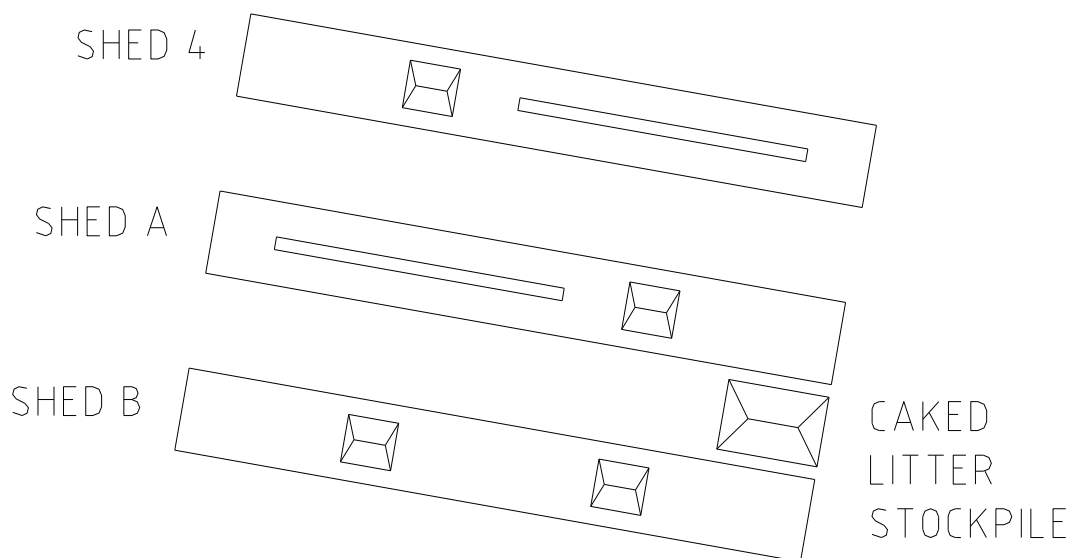


Figure 3-2: Allocation of treatments (heaps and windrows) in sheds 4, A and B.

Table 3-1: Details of treatment allocation in the three broiler sheds of the Sydney farm

Shed	Treatment	Shed Dimension	Windrow Dimensions	Heap Dimensions
4	Windrow (Win) Heap (H)	125m × 14m	H:120cm, W:200cm, L:45m	H:2.6m, Circum:4m,
A	Heap turn (HT) Windrow (Win)	120m × 14.5m	H:120cm, W:200cm, L:48m	H:2.5m, Circum:4m,
B	Heap (H) Heap turn (HT)	120m × 14.5m		H:2.8m, Circum:4m,

3.2.1 Physical measurements

The following physical measurements were conducted from the farm over the nine day period.

Farm location, shed dimensions, type and orientation. Also litter depth and sampling depths from litter prior to heaping (day 0 measurement).

Temperature distribution (spatial and temporal). Continuous temperature was monitored using iButton[®] data loggers from various sites of the litter described below:

Litter Windrow Temperature was recorded from 4 depths (0, 25, 50 and 100 cm) at two angles (90° and 45°) at 3 locations along the windrow (5 m from one end, middle and 5 m from the other end). Twelve iButtons were placed in each windrow.

Litter Heap. Temperature was recorded from four depths (0, 25, 50 and 100 cm) from three locations, again 12 buttons in each heap. During turning of the heap, the buttons were removed and then replaced following turning.

Ammonia measurement. Ammonia was measured around the heaps as well as near the machinery while it was forming piles or de-caking the litter. Ammonia was measured using a VRAE 7800 series ammonia meter. This meter had a detection range of 0–50 ppm for NH₃. On 31 October 2008, the ammonia meter was activated and taken approximately 1.2 km away from the chicken farm (and away from other obvious sources of ammonia) to perform a zero air calibration. Upon return to the farm, concentrations of ammonia were measured around the heaps and windrows in sheds 4, A and B. Because the ammonia measurements were taken in ambient air, the ammonia concentration rarely stabilised and the recorded values should be regarded as the maximum values.

Air temperature, humidity and wind speed. These were also measured using a hot wire anemometer (TSI Incorporated VelociCalc® Model 8386–M–GB).



Figure 3-3: De-caking machine used to remove caked litter from the shed after removing birds and before heaping



Figure 3-4: Using a tractor mounted blade to form the litter into heaps within the broiler shed



Figure 3-5: Heaped broiler litter in the shed; 2 heaps were formed in each shed (Shed B)



Figure 3-6: Windrow formed in the broiler shed in lieu of one of the heaps (Shed 4)

3.2.2 Litter sample collection

Litter samples were collected at days 0, 3, 6 and 9 following the application of the treatments and transported to UNE on the same day in the evening. Samples from different depths and locations were mixed and pooled by shed so that each sampling day results in 6 samples being transported to UNE for bioassay. A portion of the same samples were also collected in 100 ml jars for moisture content and pH measurements. The procedure of sampling collection was as follows:

Day 0 (all sheds) and no treatment sheds (all sampling days). Approximately 85 ml of litter from three depths (surface, 1–2 cm depth; middle, 5–6 cm depth and bottom, 10–15 cm depth; total volume ~250 ml), was collected from 40 points on a grid covering the whole shed and the samples were pooled by depth. After mixing and removal of 250 ml from each depth for measuring pH and moisture content, the remaining ~ 9 L of litter was thoroughly mixed, packed in cloth bags, surrounded with 4 L frozen chilly bricks and transported to UNE.

Day 3, 6 and 9 sampling in windrow treatments. 400 ml of litter was collected from ten sites on the surface of the each windrow (4 L), eight sites at a depth of 25 cm (3.2 L), six sites at a depth of 50 cm (2.4 L) and four sites at a depth of 100 cm (1.6 L). The litter from each depth was collected in a

separate bucket and mixed thoroughly by hand. Two subsamples of 100 ml each were collected from each of these mixtures for measuring pH and moisture content (n=3 treatments x 2 sheds x 4 and depths x 2 replicates = 48 at every time point). After taking the subsamples four buckets of litter was mixed together and the total quantity (approximately 9 L) of litter from each windrow was transported to UNE. The different volumes from each depth reflected the relative contributions to overall litter mass.

3.2.3 Bioassay for litter infectivity

The bioassay component of the experiment utilized a 3 (treatment in duplicate) × 4 (days) factorial (24 treatment combinations, corresponding to the field set up) design plus two positive and a negative control group, with a total of 27 treatment combinations. Twenty-seven isolators were therefore used for the study.

The treatment combinations were as follows:

Three litter treatment methods

- Litter heap after de-caking with no turning
- Litter heap after de-caking with turning (moving) of the heap at day 3
- Litter windrow after de-caking (approx 1 m high)

Four collection days during the litter treatment

- Day 0 post-treatment (day after destocking, immediately after de-caking)
- Day 3 post-treatment (one day after the windrow turning)
- Day 6 post-treatment
- Day 9 post-treatment

Two positive control (UNE) litters

- UNE-Dir* (litter taken directly from the animal house just before putting into the isolator)
- UNE-Tran* (UNE litter following a transport simulation)

Negative control (fresh litter)

- fresh wood shaving after sterilizing with autoclave

A total of 27 isolators were used, with 11 chickens per isolator (with the aim of collecting 10 serum samples from each at day 35 post-exposure) providing a total of 297 SPF chickens overall. There were only three composite litter samples at day 0 as the treatment were not applied yet. On all other collection days, six composite samples were used, one from each treatment application, giving us a total of 21 samples (3 + 3×6). Field samples was each placed in three isolators (21 isolators) and controls were each placed in two isolators (6 isolators) giving a total of 27 isolators.

SPF chickens were purchased in two batches. Chickens were hatched and received in two batches on two different days to limit the variation of the age of exposure onto the litter. In batch 1, 121 chickens were placed in 11 isolators to cover day 0 and day 4 litter treatments, plus the negative controls in duplicate. Four days later, a second batch (Batch 2) of 132 chickens were placed in the remaining 16 isolators to cover the day 7 and 10 treatments and two positive controls (*UNE-Dir* and *UNE-Tran*) in duplicate. We have previously shown that SPF chickens exposed to litter at day old or 7 days later show no difference in serological response to litter pathogens in experiment LT07-C-CB2. This design ensured that chickens do not differ in age by more than 5 days at time of exposure to litter. Chickens were exposed to litter by placing approximately 9 L of litter in two cat litter trays (40×30 cm each) in each isolator immediately after arrival of the litter at UNE.

3.2.3.1 *UNE-Dir and UNE-Tran litters:*

The positive control litter was produced by rearing 40 broiler chickens on a floor pen (4.5 m²). Approx 8–10 cm of pine wood shavings was laid on the floor. Chickens were fed broiler starter *ad lib* for the first 14 days then changed to broiler finisher. Normal brooding temperatures were used; starting at

35 °C at day 0 and declining by 1 °C every 2 days until a temperature of 21 °C was reached. Vaccination was performed as described in Table 3-2.

Table 3-2: Vaccine and vaccination schedule for positive controls in the experiment.

Vaccine	Source	Strain	Dose	Batch No	Age of chickens at inoculation	Route of inoculation
Vaxsafe Ris	Bioproperties	Strain V877	$>10^{2.4}$ EID50/dose	M02101	4 days	Subcut
FAV	Imugene	FAV8			8 days	Oral
Vaxsafe IBD	Bioproperties	Strain V877	$>10^{2.4}$ EID50/dose	BN IBD06271B	14 days	Eye drop
Vaxsafe ND	Bioproperties	Strain ND V4	$>10^{6.0}$ EID50/dose	BN NDV050350C	14 days	Eye drop
Vaxsafe IB	Bioproperties	IBV Ingham's strain	$10^{3.0}$ EID50/dose	BN IBI 061582A	19 days	Eye drop
Steggles CAV vaccine	Intervet Aust	Strain 3711	$10^{1.5/5}$ CID50/dose		19 days	Oral, x10 dose used

At day 31 of age, 12 chickens were bled and all chickens were removed. Eighteen litres of litter was stored in an esky with 4 L of ice and then placed in a climate control chamber where temperature was maintained from 16–35 °C as mentioned in Chapter 2 (Figure 2-1). This litter formed the *UNE-Tran* treatment used in the subsequent bioassay. Chickens of two isolators were exposed to this litter on the next day with all other litter from the field experiment. On the infection day, another 18 L of litter was collected from this broiler chickens and placed into two isolators directly forming the *UNE-Dir* treatment.

3.2.3.2 Measurements during the bioassay:

Serology

All mortalities were examined for gross pathology on post-mortem. At day 35-post exposure to litter, blood samples were collected. There were four different bleeding days for four exposure days. At day 42 of age, chickens were humanely killed (there were two kill days for two batches of chickens) and body weight recorded before disposing. Faecal samples from the cloacca were collected for coccidial oocyst counts.

Serum samples were analysed for serology for IBV (ELISA), CAV (ELISA), IBDV (ELISA) and NDV (ELISA) and FAV8 (ELISA), ILT (ELISA) and MDV at UNE. Commercial kits were used ELISA from IDEXX Laboratories Inc. (Maine, USA) for IBV, CAV, IBDV and NDV. Kits from TropBio Pty Limited (JCU, Townsville, QLD) was used for FAV/IBH and ILT. The MDV ELISA was developed at UNE using a serotype 1 (Rispen CVI988) vaccinal virus. However, the assay is not serotype specific.

Litter pH measurement:

pH was measured using a pH meter. Two and a half grams of litter samples were weighed in a screw cap plastic jar, where 50 ml of Milli-Q water was added. The content was shaken vigorously for one minute to mix thoroughly. The mixture was kept at room temperature for 30 minutes, within this waiting period the contents were shaken vigorously every five minutes. The pH meter was calibrated using standard buffers of pH 4 and 7 before reading the sample. The electrode was thoroughly rinsed in between sample readings. The instrument was calibrated with pH 7 buffer after every 4 samples.

Dry matter measurement:

Litter dry matter was determined after drying about 50g of litter at 80 °C for 48 hours.

Litter chemistry:

Dried litter samples (80 °C) were ground with a fine grinder. The ground litter was sieved through a 0.2 mm sieve and collected then sieved again through a 0.05 mm sieve and collected separately. The 0.2 mm sieved products were used for determining potassium (K), sodium (Na), phosphorus (P) and other trace elements using an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES, Carlo Erba Instruments, Strada Rivoltana, Milan, Italy) and the 0.05 mm products were used to determine carbon and nitrogen content using a Carlo Erba NA 1500 Solid Sample Analyser.

Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES):

This machine is capable of measuring total elemental analysis on particulate free liquid samples from 175 nm to 785 nm. Before analysis, the samples were subjected to sealed chamber digestion using perchloric acid (HClO₄) and hydrogen peroxide (H₂O₂) following a method described previously (Anderson and Henderson, 1986). In brief, approximately 0.2 g of litter material was weighted (keeping the record of the exact weight of the material) in a 50 ml borosilicate reagent bottle. Two ml of a mixture of a 7:3 (v/v) of HClO₄ and H₂O₂ and allowed to digest overnight at room temperature. The bottles were sealed tightly and placed into a warming oven at 80 °C for 30 minutes. After cooling a further 2 ml of H₂O₂ was added and incubated at 80 °C for one hour. A final volume of 25 ml was made by adding deionised water. The mixture at this stage contained 3.9% (0.65 N) HClO₄. The mixture was filtered through a 0.45 µm glass fibre filter before analysis through ICP-OES using full wavelength coverage for micro and macro molecules.

Carlo Erba NA 1500 Solid Sample Analysis:

200 mg of each sample was weighted into a 5×8 mm tin cups and loaded into the autosampler of the analyser. The sample was then introduced into the combustion furnace, which was maintained at 1030 °C. The sample and container melted, where tin acted as a catalyst promoting a violent reaction (flash combustion) in a temporarily oxygen enriched atmosphere. The combustion products were carried by a constant stream of ultra high purity helium through a reduction column at 500 °C and then through a filter of 2:1 magnesium perchlorate:quartz turnings, which adsorbed the water from combustion. The elemental nitrogen and carbon in the helium stream enter the chromatographic column (80 °C) for separation according to molecular weight and flow through to the Tracermass. The component beams arrived at collectors and their positive charge produced an electric current, which was amplified and passed to the data system. Both nitrogen and carbon were recorded on individual channels of the source controller and results given as %N, atom %, and %C, delta. The whole procedure was operated by the automated process of the Carlo Erbo Analyser.

3.2.4 Statistical analysis.

Analyses were performed with JMP7 (SAS Institute Inc. NC, USA). Where the chicken was the experimental unit measured, discrete data (serologically positives and negatives are coded as 1 and 0 respectively) analysis was done using a generalized linear model with a binomial link function (logistic) fitting the effects of shed, treatment and day and their 1st order interactions. All continuous data were analysed using a general linear model fitting the effects of shed, treatment, day, and depth (if appropriate) and their 1st order interactions. Least squares means and standard errors are presented.

A specific description of the analytical model is given in each subsection of the results section.

3.3 Results

3.3.1 Litter temperature

Litter temperature was recorded up to 9 days from at four depths three locations of the windrow (Win) and heap (H). The temperature profiles over time of each treatment combination are presented in Figure 3-7. Reduction of temperature at around 70–75 hour in Heap turn treatment was due to removal of temperature probes for about 7 hours during litter turning.

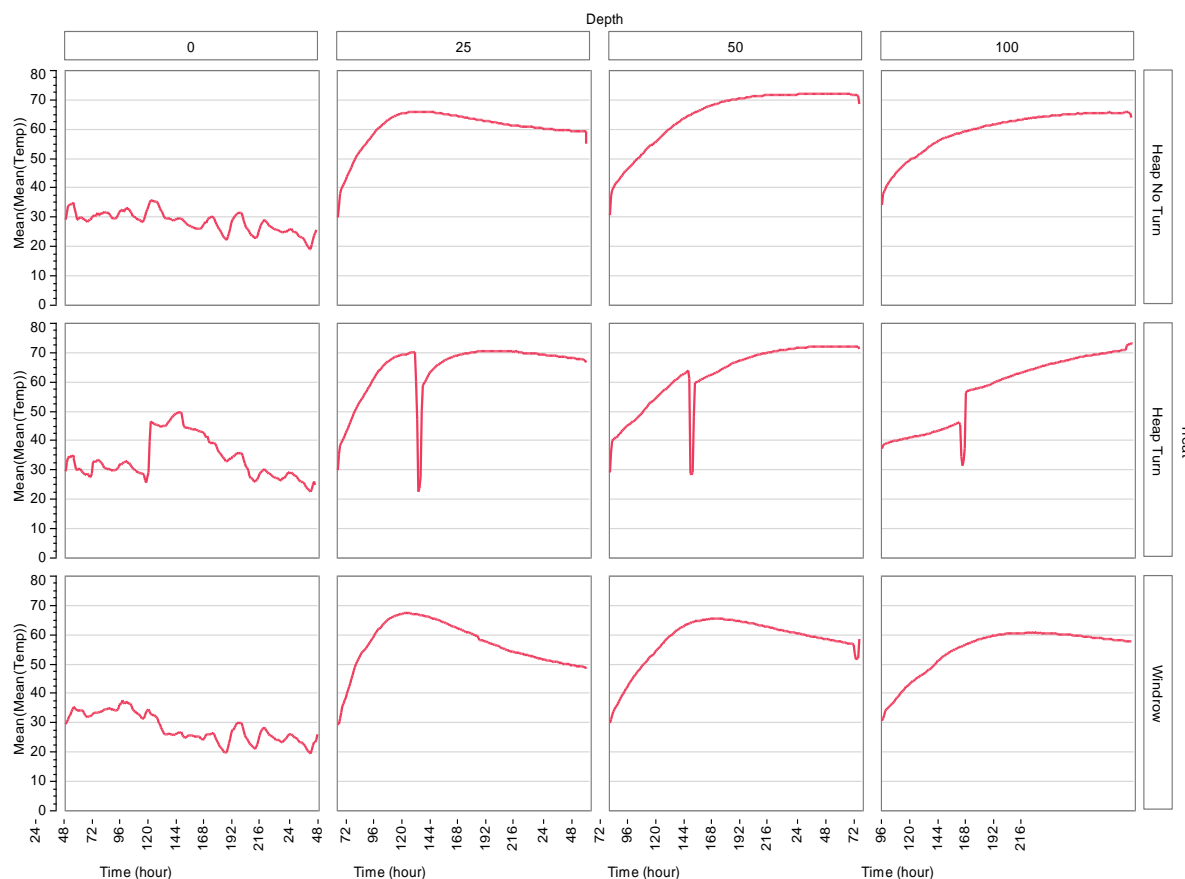


Figure 3-7: Temperature profiles (mean of 6 readings) in windrow, heap turn and heap no turn litter treatments at 0, 25, 50 and 100 cm depths. The sharp reduction in the heap turn treatment at approx. 70–75 hours was due to removal of the temperature probes during litter turning.

To compare the mean temperature between treatments, two separate analyses were conducted. In the first analysis, comparison were made up to 68 hours, before removing the iButtons for tuning. The second analysis was a comparison of temperature after the turning (75 hours onward).

Analysis 1 revealed a significant effect of shed ($P<0.001$), depth ($P<0.0001$), and treatment ($P<0.001$), on the temperature before litter turning (from 0 to 68 hours). There was a significant interaction between the effects of treatment and depth ($P<0.001$) and shed and depth ($P<0.001$). The temperature was higher in Shed B (49.84 °C) than Shed A (45.35 °C) or Shed 4 (43.92 °C). The overall temperature was highest in windrow (48.26 °C) followed by heap (46.88 °C) and heap turn (43.97 °C) at the early days post-treatment (before turning, up to 68 hours). The highest temperature was at 25 cm depth (57.44 °C), followed by 50 cm (50.25 °C), 100 cm (45.99 °C) and the surface (31.80 °C). The temperature interaction chart is presented in the Figure 3-8 (left).

After the turning (75 hours onwards), there was significant effect of shed ($P<0.001$), depth ($P<0.0001$) and treatment ($P<0.001$) with a significant interaction between the effect of treatment and depth ($P<0.001$) and shed and depth ($P<0.001$). Again, the highest temperature was in Shed B (57.52 °C)

followed by Shed A (54.39 °C) and Shed 4 (53.56 °C). Among the treatments, the temperature was highest in the heap turn (58.24 °C), followed by heap (55.45 °C) and win (51.78 °C). The highest temperature was found at the depth 50 cm (66.28 °C), followed by 100 cm (62.75 °C) and 25cm (62.58 °C) (there was no significant difference between 25 and 100 cm) and the lowest was on the surface (29.01 °C). The temperature in three treatments at various depths was presented in Figure 3-8 (right).

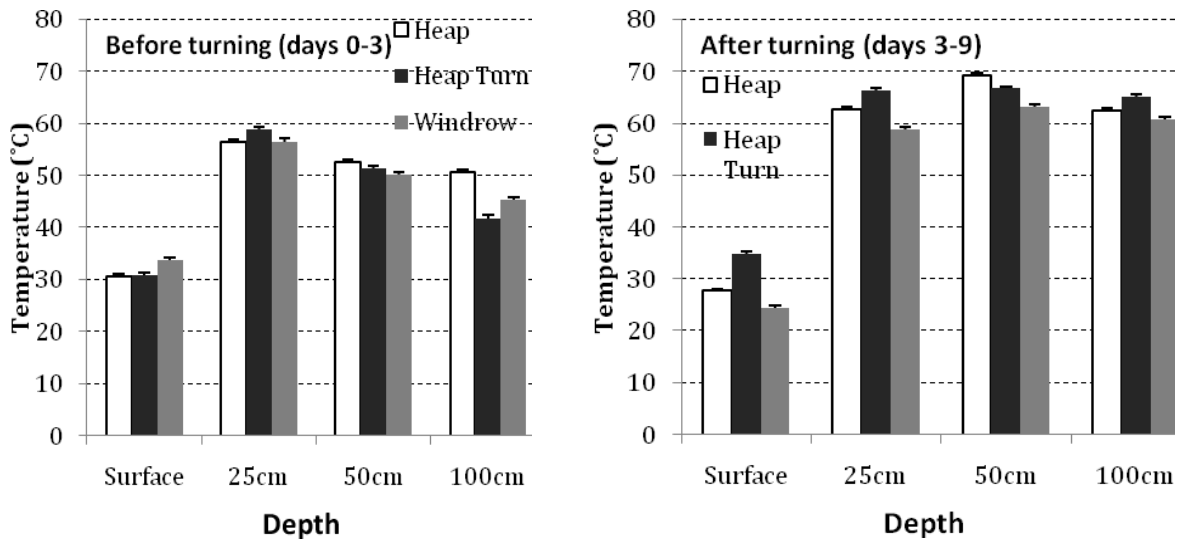


Figure 3-8: Mean (LSM±SEM) temperature at various depths in all three treatments: before (left) and after (right) turning the litter at day 3.

The most useful and interesting feature of the temperature data is the effect of treatment and depth over time. Formal analysis of the data over time is difficult, because of the factors involved. And most importantly, there was a period where temperature probes were taken out of the heap for turning; during this time true heap temperature was not recorded. Therefore the mean data representing the main effect of litter treatment and depth over time are presented in Figure 3-9 and Figure 3-10.

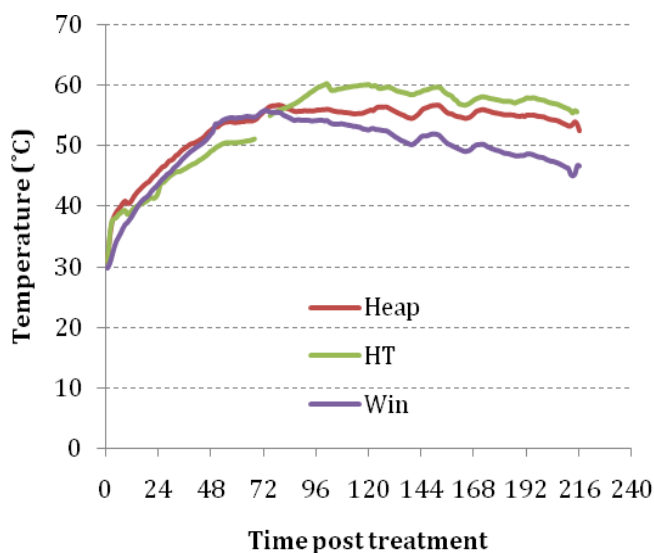


Figure 3-9: Temperature profile (mean of 24 readings) in windrow (Win), heap turn (HT) and heap treatments (all depths combined). The missing data in the HT curve at around 70–75 hours are due to removing the temperature probes during turning of the litter.

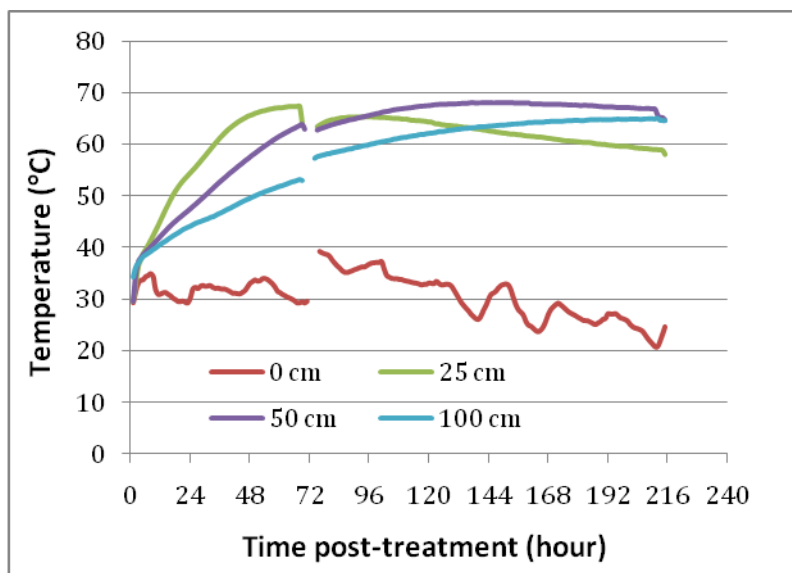


Figure 3-10: Temperature profile (mean of 18 readings) at various depths (all treatments combined). The missing data in the curves at around 70–75 hour were due to taking off the temperature probes during turning the litter in the heap turn treatment.

3.3.2 Litter pH

As mentioned previously, litter samples were collected from three depths at day 0 (before applying the treatments) and four depths at days 3, 6 and 9. In other words, the design of the litter chemistry study was not full factorial. Therefore full model analysis was not possible. The first analysis of the full data set was conducted to test the main effect of day and shed. A second analysis was conducted to explore the effect of depth on day 0 data only. The last analysis was conducted to test the effect of day, depth, treatment and shed with their interactions excluding the day 0 data.

In the first analysis, there was a significant effect of shed ($P < 0.001$) and day ($P < 0.001$) with a significant interaction between the main effects ($P < 0.001$). The lowest pH was observed at day 9 (8.49), which was significantly lower than the pH of days 0 (8.72), 3 (8.74) and 6 (8.63). Shed A (8.78) had higher pH than Shed B (8.54) and 4 (8.60). The lowest pH was recorded in the Shed B litter at day 0 (Figure 3-11). There was no significant effect of depth within day 0 data (second analysis).

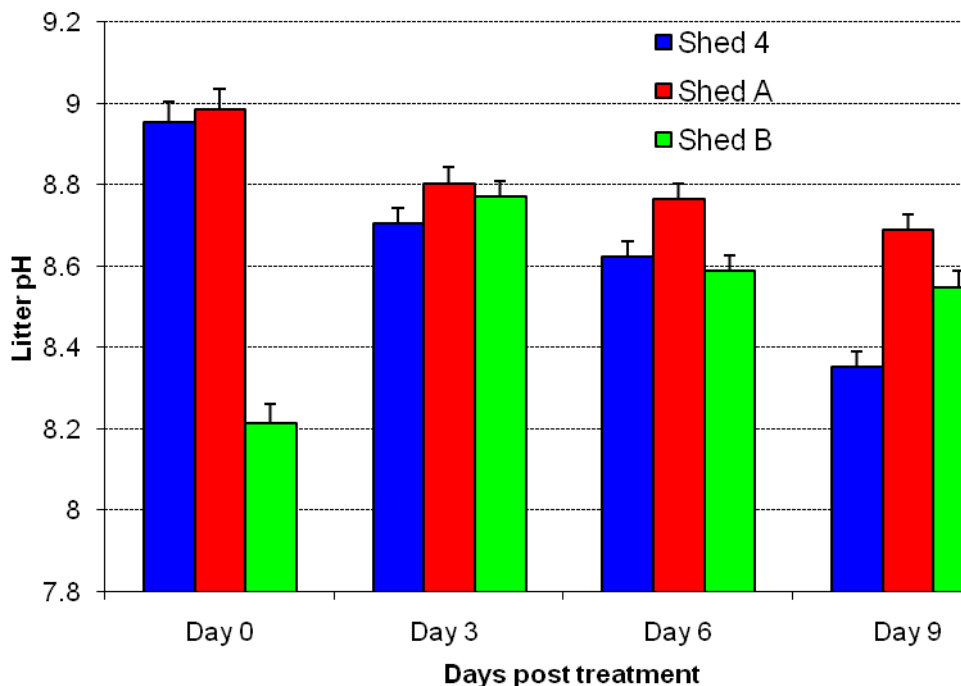


Figure 3-11: Litter pH interaction plot (LSM±SEM) for the effects of shed and day of sampling. All depths combined.

Analysis excluding day 0 revealed that there was significant effects of day, shed, treatment and depth ($P < 0.001$). Higher pH was recorded on day 3 (8.70) than day 6 (8.61) followed by day 9 (8.49). The pH of Shed 4 (8.48) was lower than Sheds A (8.69) and B (8.63). Windrow had highest pH (8.69) followed by heap (8.62) and heap turn (6.50). The surface litter had higher pH (8.82) than all other depths (8.51–8.55) irrespective of treatments (Figure 3-12).

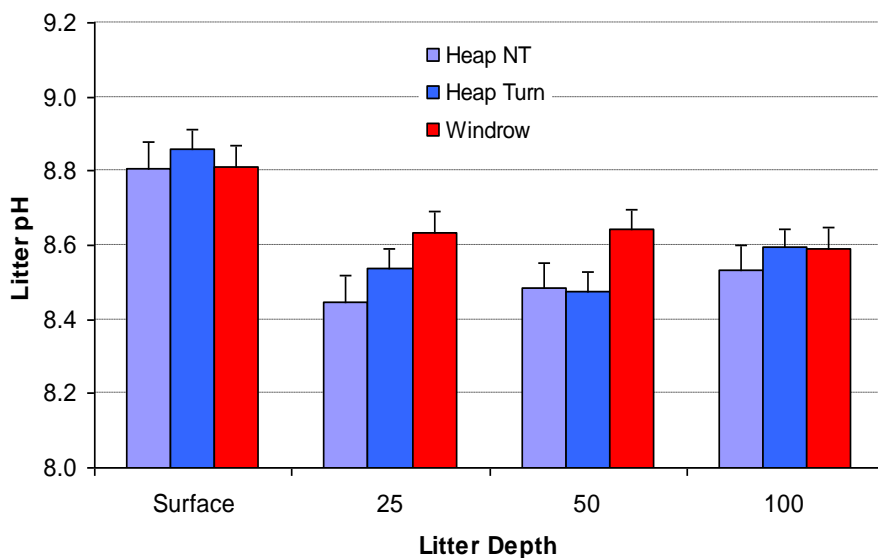


Figure 3-12: Litter pH (interaction plot (LSM±SEM) for the effects of litter depth and treatment with day 0 data (pre-treatment) excluded.

3.3.3 Litter dry matter

As mentioned earlier, litter samples were collected from three depths at day 0 (before applying treatments) and four depths at days 3, 6 and 9. In other words, the design of the litter collection was not full factorial. Therefore full model analysis was not possible for dry matter data. The first analysis of the full data set was conducted to test the main effect of day and shed. A second analysis was conducted to explore the effect of depth on day 0 data only. The last analysis was conducted to test the effect of day and depth, treatment and shed with their interactions excluding day 0.

There was no significant main effect of day ($P < 0.08$) or shed ($P = 0.16$) on the litter dry matter content, however, there was a significant interaction between the effects of shed and day ($P < 0.05$). The dry matter was lower at day 0 from Shed B whereas at day 9 lower DM was observed in the litter from Shed 4 (Figure 3-13). Overall, there was trend of drying litter over time (day).

Within day 0 sample, there was a significant effect of depth with a higher DM in the surface litter (79%) than bottom litter (73%), whereas the DM of the middle litter (75%) was not significantly different either from the bottom or surface litter.

Analysis of data excluding the day 0 measurements, there was a significant effect of depth ($P < 0.001$) but not day ($P = 0.95$), treatment ($P = 0.48$) or any of their interaction. The surface litter (82%) had higher DM than any other depths (76–79%).

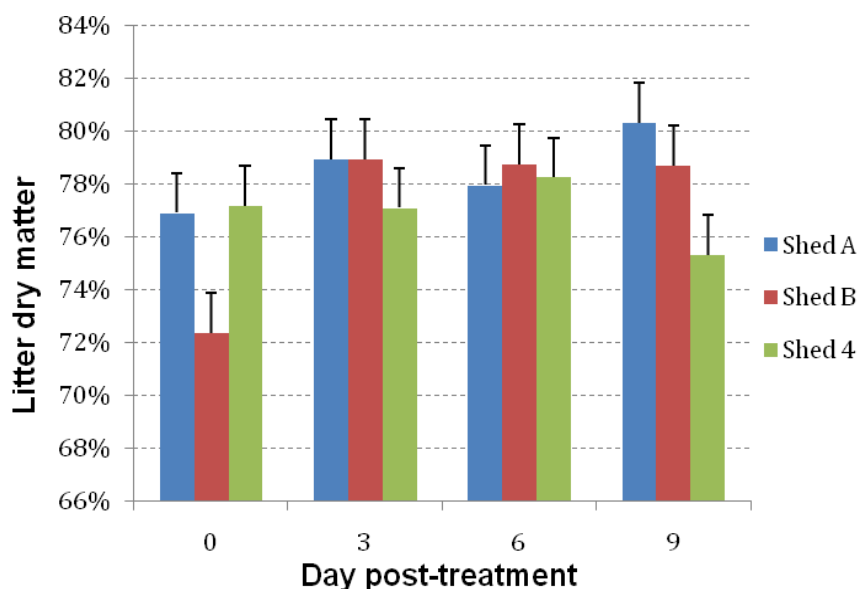


Figure 3-13: Litter dry matter interaction plot (LSM±SEM) for the effects of shed and day of sampling. All depths combined.

3.3.4 Litter nitrogen content

The litter nitrogen data were analysed using the same statistical models as pH and dry matter.

There was significant main effects of day ($P < 0.001$) and shed ($P < 0.003$) but not their interaction on the litter nitrogen content. Nitrogen content was lower in Shed A (3.7%) than Sheds B (3.8%) and 4 (3.8%). Day 6 nitrogen content was lower than other days (Figure 3-14). There was a significant effect of depth ($P < 0.03$) on nitrogen content within day 0 data with lower nitrogen in the surface litter (3.6%) than middle (3.9%) and bottom litter (3.8%).

Analysis excluding the day 0 data revealed significant effects of day ($P < 0.004$) and depth ($P < 0.001$) but not treatment ($P = 0.22$) on the nitrogen content in the litter. No interaction between any of the main

effect was significant. Again the day 6 nitrogen content was lower than other days and surface litter was lower than the litter from any other depths.

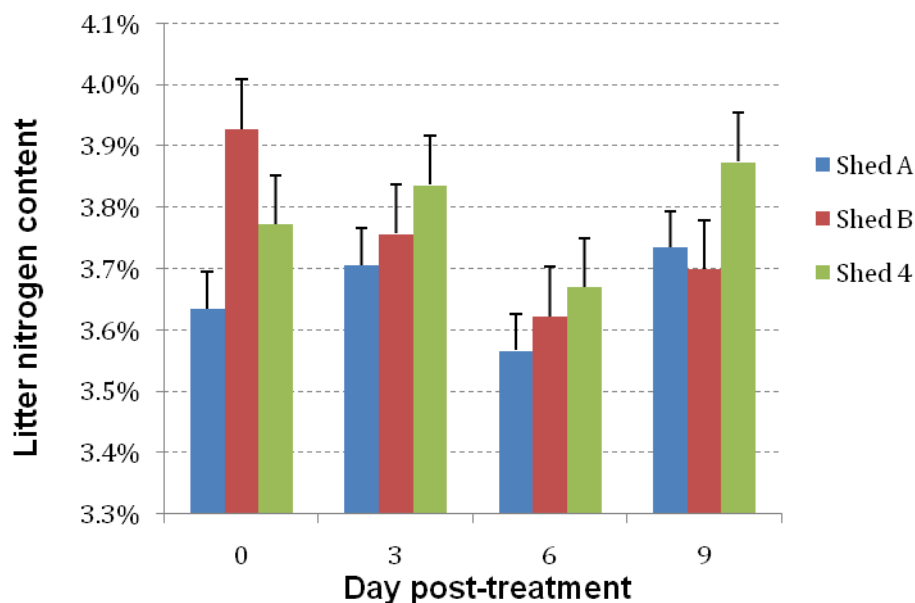


Figure 3-14: Litter nitrogen (LSM±SEM) content at days 0, 3, 6 and 9 post-litter treatment from three different sheds (all depths and treatments combined).

3.3.5 Litter carbon content

The litter carbon data were analysed using the same model as pH, dry matter and nitrogen.

There was significant main effects of day ($P<0.001$) and shed ($P<0.004$) on the litter carbon content with a significant interaction between the effects of day and shed ($P<0.001$). The carbon content was lower in Shed B (36.2%) than Shed 4 (37.6%), whereas Shed A (36.7%) carbon content was not significantly different from either Sheds B or 4. Day 0 carbon content was lowest (35.7%) followed by day 6 (36.3%), 9 (37.4%) and 3 (38.0%). day 0 litter from Shed B had the lower carbon content than any other treatment combinations (Figure 3-15). There was a significant effect of depth ($P<0.001$) on carbon content within day 0 data with lower carbon in the bottom litter (31.4%) than middle (36.8%) and surface litter (38.9%).

Analysis excluding the day 0 data revealed significant effects of day ($P<0.001$) and treatment ($P<0.001$) but not depth ($P=0.12$) on the litter carbon content. No interaction between any of the main effect was significant. Day 6 had lower carbon content than other days and heap turn (36.9%) had lower carbon than heap (38.0%) without any difference between heap turn and win (37.4%) and win and heap.

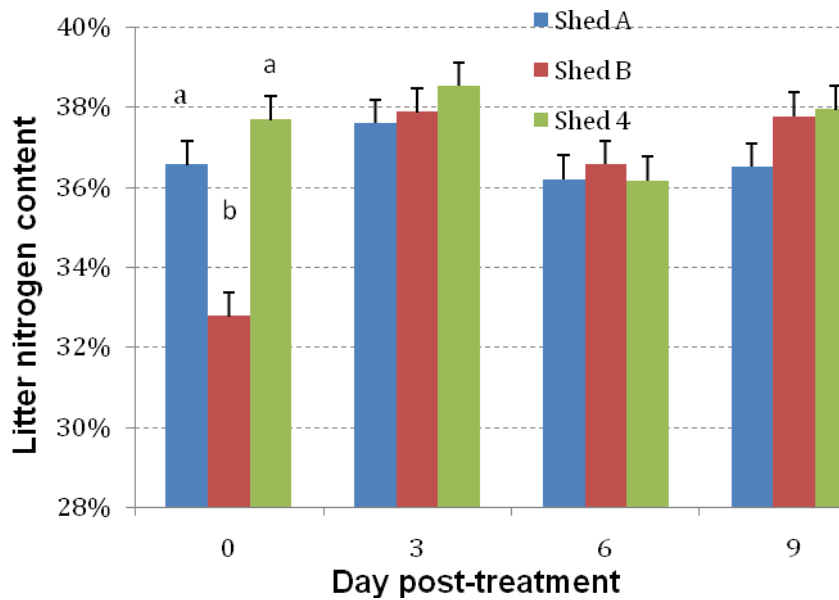


Figure 3-15: Litter carbon (LSM±SEM) content at days 0, 3, 6 and 9 post-litter treatment from three different sheds (all depths and treatments combined).

3.3.6 Association between litter chemistry variables

There was a significant positive association between the dry matter content and litter pH ($R^2=0.33$, $P<0.001$) and negative associations between dry matter and nitrogen content ($R^2=0.179$, $P<0.001$), pH and nitrogen content ($R^2=0.18$, $P<0.001$) but not between dry matter and carbon content ($R^2=0.01$, $P=0.15$), pH and carbon ($R^2=0.04$, $P=0.10$) and carbon and nitrogen content ($R^2=0.05$, $P=0.32$). The litter pH increases with increase of dry matter in the litter and pH decreases with reduction of nitrogen in litter (Figure 3-16).

Chapter 3 – Sydney litter experiment. Effect of in-shed partial composting of litter on the inactivity of viral infectivity

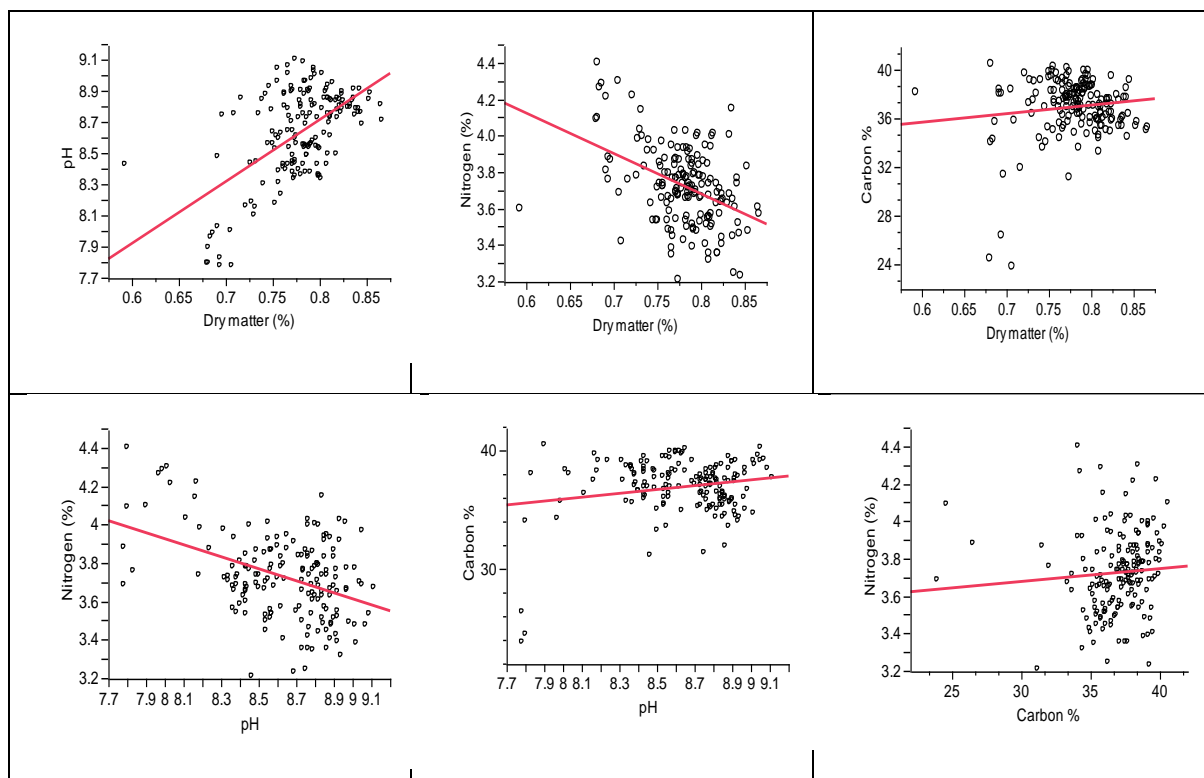


Figure 3-16: Association between key litter chemistry variables.

3.3.7 Air ammonia measurements

3.3.7.1 Ammonia measurements in Shed B

Ammonia concentration was measured in Shed B around the heap on the eastern end of the shed according to Table 3-3 and Figure 3-17. Measurements were undertaken at 9.20 am. At this time, air temperature was 26.5 °C and relative humidity was 66.3%.

Table 3-3: Sampling positions and measured ammonia concentration around heap in shed B

Sampling position	Height from ground (m)	Distance from heap (m)	Measured ammonia concentration (ppm)
A (upwind)	1.0	1.0	0–2
B (downwind)	0.3	0.3	20–26
C (downwind)	1.0	1.0	8–10

Chapter 3 – Sydney litter experiment. Effect of in-shed partial composting of litter on the inactivity of viral infectivity

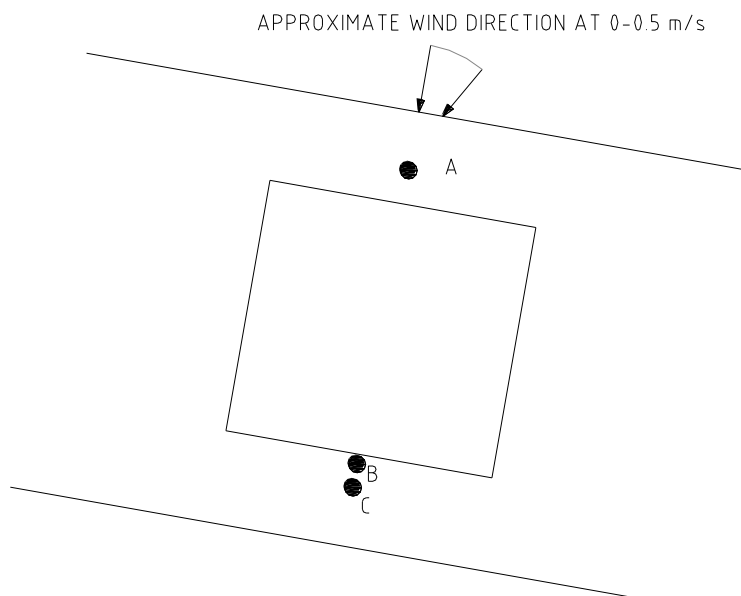


Figure 3-17: Approximate wind direction and ammonia measurement points for Shed B (eastern heap)

3.3.7.2 Ammonia Measurements in Shed A

Ammonia concentration was measured in Shed A around the heap on the eastern end of the shed according to Table 3-4 and Figure 3-18. Measurements were undertaken at 9.30 am. At this time, air temperature was 28.7 °C and relative humidity was 61%. Measurements were undertaken while the heap was being formed with the tractor.

Table 3-4: Sampling positions and measured ammonia concentration around heap in shed A

Sampling Position	Height from ground (m)	Distance from heap (m)	Measured ammonia concentration (ppm)
A (upwind)	1.0	2.0	0-2
B (downwind)	1.0	2.0	30-35

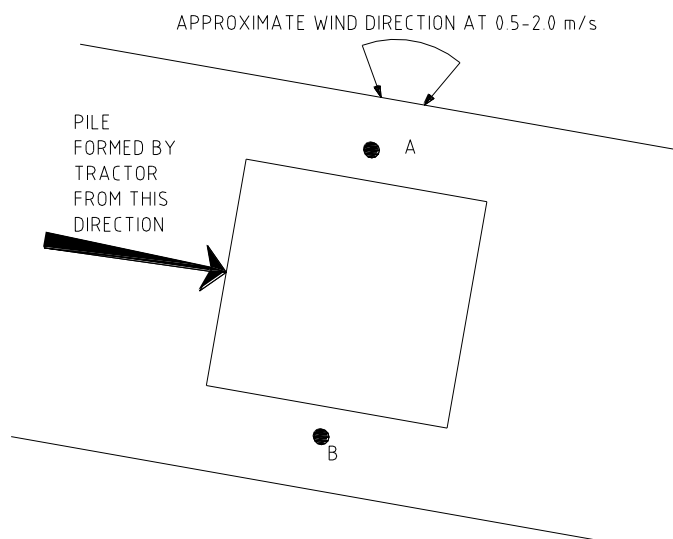


Figure 3-18: Approximate wind direction and ammonia measurement points for Shed A (eastern heap)

3.3.7.3 Ammonia Measurements in Shed 4

Ammonia concentration was measured in Shed 4 while the de-caking machine was being operated. Measurements were recorded according to Table 3-5. Measurements were undertaken at 9.45 am. At this time, air temperature was 29 °C. Wind speed and direction were similar to those measured in shed A.

Table 3-5: Sampling positions and measured ammonia concentration around heap in shed 4

Sampling position	Comments	Height from ground (m)	Measured ammonia concentration (ppm)
Upwind side of the shed	Measurement not influenced by decaking operation	1.2	0–1
Middle of the shed		1.2	0–4
Downwind side of the shed		1.2	0–10
Behind de-caking machine	During de-caking operation	1.0	steady value 10–15 maximum peak 28
Beside de-caking machine	In dust plume on downwind side	1.0	30–38

3.3.7.4 Ammonia Measurements in around the caked litter stockpile

Ammonia concentration was measured near the caked litter stockpile. Caked litter was being deposited at this time, however no machinery was actively disturbing the pile during ammonia measurement.

Table 3-6: Sampling positions and measured ammonia concentration around heap in shed 4

Sampling position	Height from ground (m)	Distance from pile (m)	measured ammonia concentration (ppm)
Upwind position	1.2	2	0
Downwind position	0.3	0.3	7–10
Downwind position	1.0	1.0	2

3.3.7.5 Other ammonia measurements

On several occasions, a small amount of litter on the surface of the heaps was disturbed (approximately 0.15 m by 0.15 m area and approximately 0.15 m deep). When the ammonia meter was placed near this disturbed litter and within approximately 0.1 m of the litter surface, ammonia concentration rapidly increased until the upper limit of the ammonia meter was reached (50 ppm).

3.3.8 Litter infectivity

The serological data were coded as 1 for positive and 0 for negative. Analyses were conducted using a generalized linear model with a binomial link function (logistic) fitting the effects of treatment and day and their interaction. Where a significant number of animals was not positive, only raw data are presented without any analysis. Serological titres (where applicable) were analysed using general linear model of analysis of variance.

As the design of the experiment was not full factorial because at day 0 only one sample was collected from each shed before the application of litter treatments. At days 3, 6 and 9, samples were collected from each treatment. At day 0 there was no effect of treatment. Therefore analysis was done separately to explore the effect of day in the full data set, whereas the effect of treatment and day and their interaction were explored in the data from days 3, 6 and 9 (excluding day 0 data). To explore the effect of transportation simulation (*UNE-Dir* versus *UNE-Tran* litters) Chi-square analysis was used.

3.3.8.1 Marek's disease virus (MDV)

When analysed using a full model of treatment and day with their interaction (excluding the two UNE litters and control), there was a significant effect of day ($P < 0.001$) but not treatment ($P = 0.99$) and their interaction ($P = 0.31$) on the MDV response. There was no difference in MDV response in windrow and heap treatment and there was no significant advantage of turning of the heap (Table 3-7). MDV was inactivated gradually over time, however by day 9, litter retained significant MDV infectivity (Figure 3-19).

There was no significant difference in MDV response between the two UNE litter ($P = 0.74$), suggesting no effect of transportation simulation on the MDV. There was a small proportion of control chickens were MDV positive with low titre, which are possibly false positives.

Table 3-7: Infectivity of MDV in litter following heaping or windrowing up to 9 days

Treatment	Day	Positive	Negative	%
Control		2	15	12
Day 0 (Pre-treatment)	0	28	1	97
Windrow	3	18	0	100
	6	8	12	40
	9	7	13	35
Heap turn	3	18	1	95
	6	12	7	63
	9	8	11	42
Heap	3	17	2	89
	6	14	6	70
	9	8	11	42
UNE-Dir		13	7	65
UNE-Tran		14	6	70

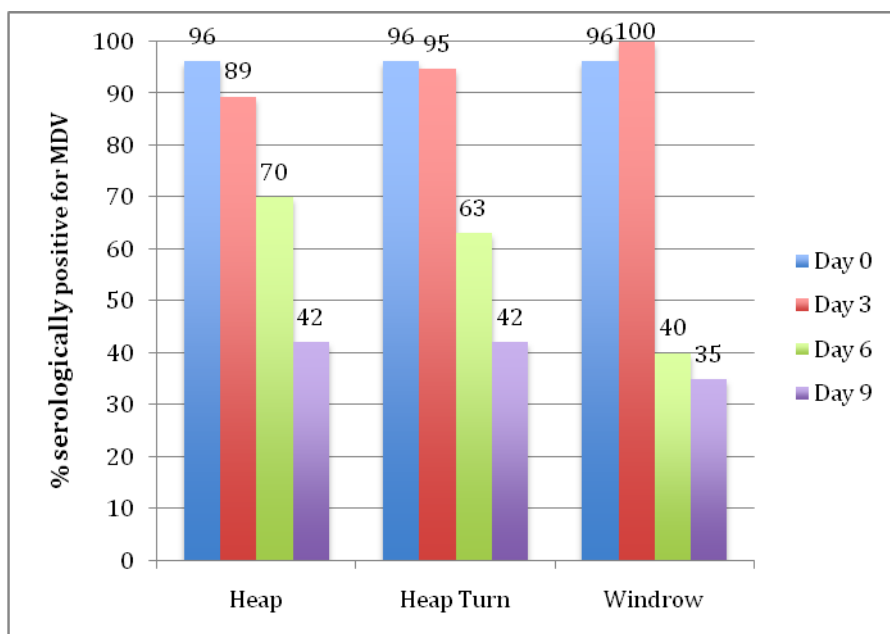


Figure 3-19: Percentage of chickens serologically positive for MDV at day 35 post exposure to litter. Interaction plot of effects of treatment and day of litter collection post heaping.

Full model analysis of MDV titre (excluding two UNE and controls) revealed a significant effect of Day ($P < 0.001$) but not Treatment ($P = 0.32$) and their interaction ($P = 0.22$). The titre was decreasing with the day. There was no difference in the titres of *UNE-Dir* and *UNE-Tran* treatments. The MDV titre of the Control group was significantly lower than any other treatments (Figure 3-20).

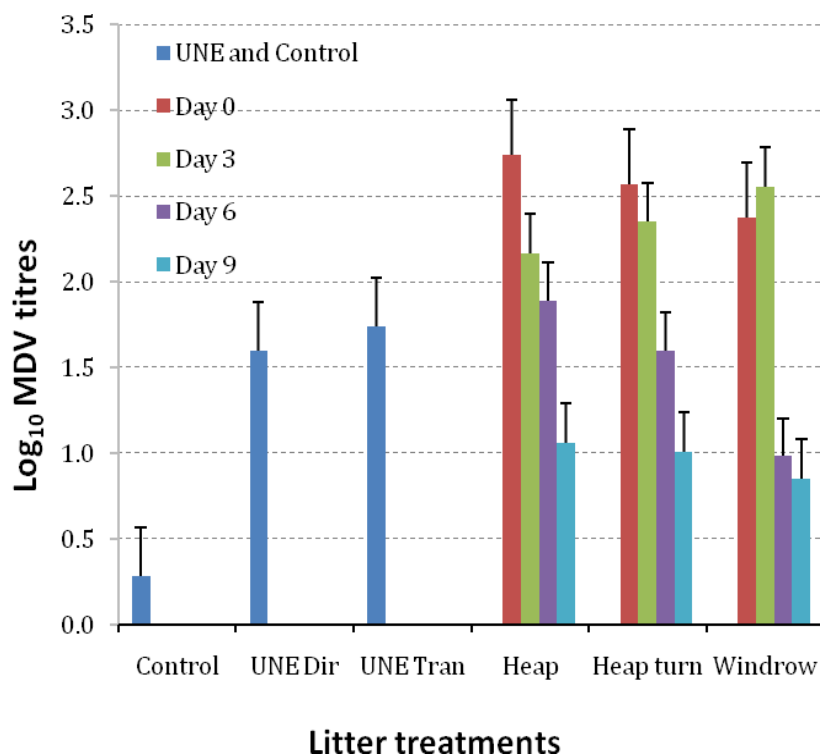


Figure 3-20: Log₁₀ MDV titres (LSM±SEM) by treatment and day post heaping.

3.3.8.2 Fowl adenovirus/inclusion body hepatitis (FAV)

There was a significant effect of day ($P < 0.001$) but not treatment ($P = 0.99$) on the FAV response and there was a significant interaction between the effect of day and treatment ($P < 0.02$). The survival of FAV in litter was decreasing over time, in most cases up to zero survival by day 3 except in few cases where FAV survived well up to day 9 (Table 3-8, Figure 3-21). No FAV was detected in the UNE and control chickens.

Table 3-8: Infectivity of FAV in litter following heaping or windrowing up to 9 days

Treatment	Day	Positive	Negative	%
Control		0	17	0
Day 0 (Pre-treatment)	0	20	7	74
Windrow	3	0	13	0
	6	1	21	5
	9	0	20	0
Heap turn	3	0	19	0
	6	0	14	0
	9	8	13	38
Heap	3	0	18	0
	6	0	11	0
	9	0	17	0
UNE-Dir		0	18	0
UNE-Tran		0	18	0

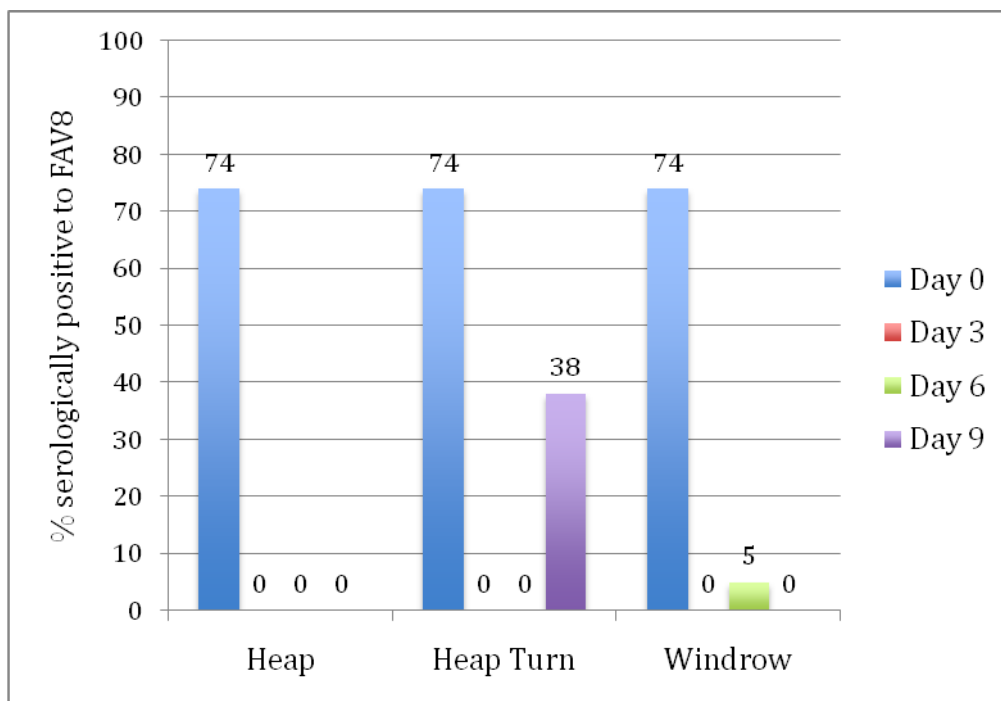


Figure 3-21: Percentage of chickens serologically positive for FAV at day 35 post exposure to litter. Interaction plot of effects of treatment and day of litter collection post heaping.

3.3.8.3 Chicken infectious anaemia virus (CAV)

There was a significant effect of day ($P < 0.001$) and treatment ($P < 0.01$) on the CAV response with a significant interaction ($P < 0.02$) between the main effects. There was lower CAV response in heap (9%) than heap turn (39%) and windrow (33%). Day 0 (97%) has much CAV response than days 3 (48%), 6 (29%) and 9 (3%). The interaction was significant because of slower reduction in CAV response Windrow than other two treatments (Table 3-9, Figure 3-22). There no chicken was positive for CAV in the control and two UNE litters.

Table 3-9: Infectivity of CAV in litter following heaping or windrowing up to 9 days

Treatment	Day	Positive	Negative	%
Control		0	17	0
Day 0 (Pre-treatment)	0	28	1	97
Windrow	3	7	11	39
	6	10	10	50
	9	2	18	10
Heap turn	3	16	3	84
	6	6	13	32
	9	0	19	0
Heap	3	4	15	21
	6	1	19	5
	9	0	19	0
UNE-Dir		0	20	0
UNE-Tran		0	20	0

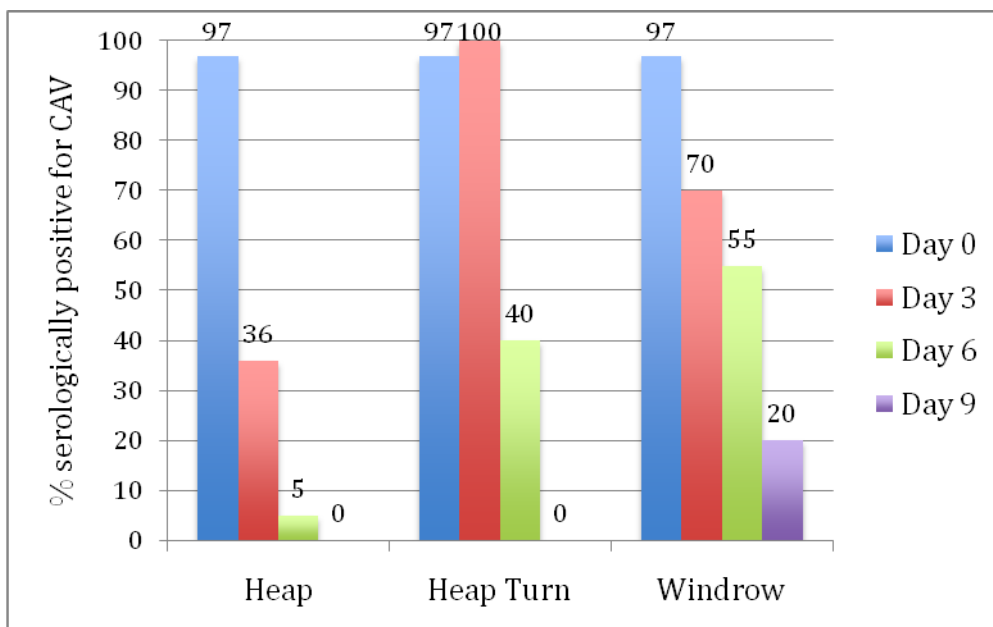


Figure 3-22: Percentage of chickens serologically positive for CAV at day 35 post exposure to litter. Interaction plot of effects of treatment and day of litter collection post heaping.

3.3.8.4 Infectious bursal disease virus (IBDV)

There was a significant effect of treatment ($P < 0.005$) on the IBDV response with higher IBDV positive chicken in Heap and Heap turn treatment than Windrow. There was no significant difference in IBDV response between *UNE-Dir* and *UNE-Tran* litters ($P = 0.20$). All control chickens were negative IBDV. The effect of day was also significant on the IBDV response, higher CAV positive chickens were found on Day 0 (21%) and Day 6 (28%) than Day 3 (7%) and 9 (3%). There was no particular trend in the CAV in the litter due to the application of treatments. However, it has been observed that CAV is decreasing over time, but not in all treatments, possible there were some spots on the surface of the litter where virus stays infective and in other places of heap or windrow inactivated over time (Table 3-10, Figure 3-23).

Table 3-10: Infectivity of IBDV in litter following heaping or windrowing up to 9 days

Treatment	Day	Positive	Negative	%
Control		0	18	0
Day 0 (Pre-treatment)	0	7	26	21
Windrow	3	1	19	5
	6	1	19	5
	9	0	20	0
Heap turn	3	1	20	5
	6	5	15	25
	9	2	18	10
Heap	3	2	17	10
	6	11	9	55
	9	0	20	0
<i>UNE-Dir</i>		3	17	15
<i>UNE-Tran</i>		1	19	5

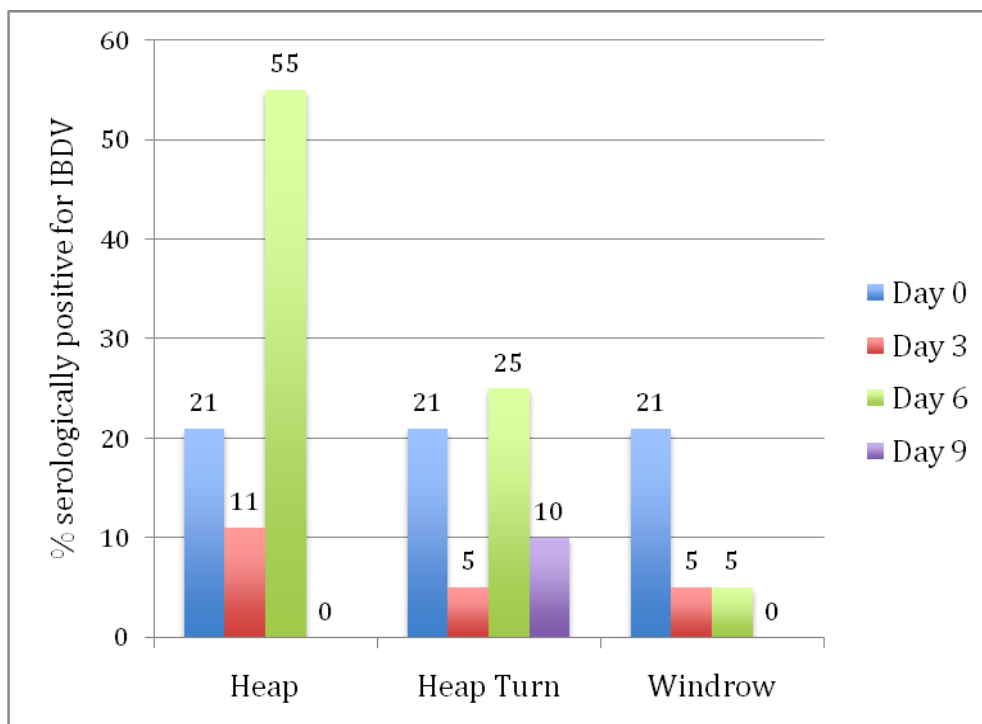


Figure 3-23: Percentage of chickens serologically positive for IBDV at day 35 post exposure to litter. Interaction plot of effects of treatment and day of litter collection post heaping.

3.3.8.5 Infectious bronchitis disease virus (IBV)

Only one litter was positive for IBV antibody (*UNE-Dir*, 50%), which indicates the virus does not survive well in the litter (Table 3-11).

Table 3-11: Infectivity of IBV in litter following heaping or windrowing up to 9 days

Treatment	Day	Positive	Negative	%
Control		0	18	0
Day 0 (Pre-treatment)	0	0	33	0
Windrow	3	0	20	0
	6	0	20	0
	9	0	20	0
Heap turn	3	0	21	0
	6	0	20	0
	9	0	20	0
Heap	3	0	19	0
	6	0	20	0
	9	0	20	0
<i>UNE-Dir</i>		10	10	50
<i>UNE-Tran</i>		0	20	0

3.3.8.6 Newcastle disease virus

No chicken was positive for NDV antibody.

3.3.8.7 *Coccidial oocyst count*

Coccidial oocyst counts decreased with litter treatment. No surviving oocysts were detected beyond day 3 of litter treatment. These data were not analysed statistically, and raw data are presented in Table 3-12.

Table 3-12: Coccidial oocyst count (per gram of faeces) from faecal sample collected from isolator at the termination of the experiment (day 42 of chicken age).

Shed	Treatment	Day 0	Day 3	Day 6	Day 9
A	Heap Turn	241,000	0	0	0
	Windrow		0	0	0
B	Heap NT	42,500	0	0	0
	Heap Turn		1,000	0	0
4	Heap NT	16,000	0	0	0
	Windrow		0	0	0
UNE	Tran	0			
	Dir	0			
Neg Control	Nil	0			

3.3.9 Mortality

Only three chickens died or were euthanised during the whole experimental period without any significant effect of treatment ($3/297 = 1\%$ mortality). These were at days 1 and 31 of age with no visible lesions, and at day 41 an accidental death due to a chick being caught behind a feeder.

3.3.10 Body weight (BW)

Body weight data were analysed separately for the effects of day and treatment. No separate treatment was not available for day 0 (prior to the application of the treatment), therefore a full model analysis was not possible. A full model analysis was done excluding the day 0 data.

There was a significant effect of day ($P < 0.001$) with no significant effect of treatment ($P = 0.12$) on the body weight. The BW was lower in day 0 (453 ± 13 g) than all other days (506 – 536 g) and negative control (547 ± 17 g) (Figure 3-24). Chickens reared on the fresh litter (day 0, prior to the application of any treatment) straight from the broiler shed had negative impact on the growth of chicken, possibly due to heavy load of pathogens.

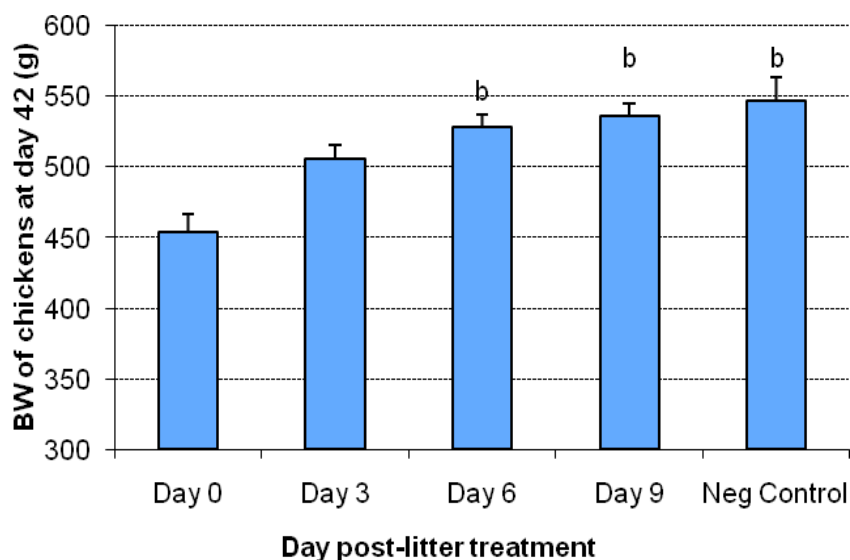


Figure 3-24: Body weight (LSM±SEM) of chickens at day 42.

3.4 Discussion and conclusions

This experiment has reported for the first time we are aware of, the temperature profile at various depths of litter heaps and windrows over time under field conditions. The temperatures from 25 cm to 100cm depths reached 60 °C by day 4 and continued to increase and plateau (50 and 100 cm) or decrease (25 cm) thereafter. However, the temperature on the surface of the litter maintained at around 31–35 °C.

With regards our specific hypotheses, the findings are summarised as follows:

Hypothesis 1. Infectivity of litter for viruses and coccidia will be reduced following heaping or windrowing. This hypothesis was supported for CAV, FAV, MDV and coccidia for which there were clear reductions in the infectivity of litter with heaping and windrowing treatments. Coccidial oocysts were inactivated quickly, with no evidence of litter transmission at day 6 onward. This is consistent with the report that high temperature (~65 °C) in the presence of ammonia inactivates coccidial oocysts within hours (McDougald and Fitz-Coy, 2008). However, it is not clear in the study whether this inactivation of pathogen occurred due litter treatments or with effect of time alone, because there was no control litter treatment (without heaping or windrowing in the shed).

CAV and FAV are relatively environmentally resistant viruses (Schat and Woods, 2008; Adair and Fitzgerald, 2008) and the reduction in infectivity was greatest for the heap treatment without turning. This treatment resulted in no detectable infectivity by day 9. Turning the heaps appeared to reduce the rate of decline in infectivity and resulted in some infectivity appearing at day 9 for FAV. MDV is also an environmentally resistant virus. There was loss of MDV infectivity over time equally in all treatments and it was not clear whether the inactivation was due to time alone or due to litter treatment. By day 9, all litters still retained significant infectivity.

The results for IBDV are inconclusive as initial litter infectivity was low but again positive samples were observed as late as day 9 demonstrating the well-documented environmental resistance of this virus. The appearance of IBV in two samples at day 7 post-treatment was unexpected as the virus is fragile and we have previously found that it does not transmit effectively on litter particularly after transportation (Islam et al., 2009).

Hypothesis 2. Infectivity will be reduced more in treatments generating greater temperatures. Temperature up to day 3 (before turning) was highest in windrow (~49 °C) followed by heap (~47 °C) and heap turn (~44 °C) treatments. However, after turning on day 3, on average, highest temperatures were achieved in heap turn (~57 °C) followed by heap (~55 °C) and windrow (~43 °C). As the bulk of the time was post turning, these latter values represent the order for overall mean temperatures. The higher temperatures in the heap treatment were associated with significantly greater inactivation of CAV, but this was not true for MDV, IBDV or coccidia.

Hypothesis 3. Heaping will be more effective than windrowing. As noted above the higher temperatures in the heap treatment were associated with significantly greater inactivation of CAV, but this was not true for MDV, IBDV or coccidia in heaps compared to windrows.

Hypothesis 4. Turning will lead to increased inactivation. There was no evidence of improved pathogen inactivation with turning in this experiment. This is somewhat unexpected as temperatures on the surface of the heap remained low, around 31–35 °C and it could reasonably be expected that mixing this surface material in with the heap at day 3 would lead to greater inactivation overall.

Hypothesis 5. Most of the benefits in terms of pathogen inactivation will be seen by day 3. This hypothesis is supported for coccidia and FAV but not CAV, MDV or IBDV.

Hypothesis 6. Ammonia measurements during litter treatment will fall within acceptable levels (<25 ppm). This hypothesis is accepted apart from downwind locations very close to the heap (0.3 m) or during actual de-caking and heap forming operations. Continuous measurement by the ammonia meter enabled assessment of peak and short-term steady values for airborne ammonia concentration.

Chapter 3 – Sydney litter experiment. Effect of in-shed partial composting of litter on the inactivity of viral infectivity

At all times, the sidewall curtains on the sheds were open and a slight breeze was blowing (measured to be 0–2.0 m/s on the inside of the shed).

On the upwind side of the shed or litter heaps, ammonia concentration rarely exceeded 2 ppm even when the exhaust air from upwind sheds were blowing in. On the downwind side of the heaps, ammonia concentration reached approximately 26 ppm when positioned within 0.3 m of the heap. Once the meter was repositioned to be 1.0 m from the heap, ammonia concentration reduced to approximately 10 ppm. During de-caking and heap forming operations, disturbance of the litter caused the ammonia concentration to increase to approximately 35–38 ppm; however, these peaks only occurred for very short periods.

The Australian Government has recommended safe workplace exposure limits for ammonia (Australian Safety and Compensation Council, 1995). These are given in terms of an eight hour time weighted average (TWA) exposure of 17 mg/m³ (approximately 25 ppm) and a fifteen minute short term exposure limit (STEL) of 24 mg/m³ (approximately 35 ppm). When compared to safe workplace levels, the concentration of ammonia measured at this farm occasionally exceeded the levels listed for the time weighted and short term exposure limits (TWA and STEL). On this occasion, no attempt was made to measure the actual exposure for the grower (or machinery operator) for the durations specified by TWA and STEL. However, because the concentration of ammonia exceeded those corresponding to TWA and STEL, there is a risk of excessive exposure and growers should assess the risk of excessive ammonia exposure for themselves and workers, especially while de-caking litter or forming, turning or spreading the heaps.

Other findings and conclusions. There were not any drastic changes in the litter chemistry (pH, dry matter, carbon and nitrogen contents) due heaping or windrowing over time up 9 days.

Two limitations to this study are apparent. The first is that the outcome is dependant on the range of pathogens present on the site being tested. Thus we have limited or no data on the effectiveness of the litter treatments against ILTV and IBDV due to the low level of initial pathogen load in the samples. In future it may be preferable to work with litter that has been deliberately contaminated by chickens harbouring a wider range of pathogens. A second limitation is the appearance of unexpected increases in infectivity of samples over time. This occurred in some cases with FAV, IBDV and IBV but were mostly small. It probably reflects sampling variation despite the rigorous sampling procedure used (10 sites and 4 depths per windrow or heap). A localised source of surviving virus in the litter may only be sampled by chance on one of the sampling days. A small number of weak positives following a series of negative results may also represent false positives. Expressing the data in terms of mean titre, rather than ratio of positive to negative may reduce this effect.

Despite this, the study has provided a clear demonstration of the efficacy of litter heaping and windrow treatments in reducing litter transmission of CAV, FAV and coccidia.

The survival of viral pathogens and coccidial oocysts decreased significantly in the treated (heaped or windrowed) litter over time, with a complete loss of infectivity of coccidia by day 6. This study showed that IBV and NDV do not survive in the poultry litter well and these two viruses are not litter transmissible.

The infectivity of MDV and CAV also decreased over time due to litter treatments; however, these viruses retained infectivity by day 9 significantly. The test survival of IBDV in the litter was not conclusive, because of very low infectivity of the field litter with the virus. However, the virus still retained infectivity at day 9.

4 Effect of in-shed partial composting of litter on the inactivation of viral infectivity

Experiment LT09-C-CB5 & CB6

Start: 12/05/09

Completion: 01/07/09

AEC: UNE AEC09/001

4.1 Introduction

This was the second field experiment to determine effectiveness of litter treatment for the inactivation of poultry pathogens. In the Sydney experiment, effectiveness of two litter treatments, heap and windrow for the inactivation of pathogens were compared. As mentioned in the previous chapter, the preparation of one or two large heap(s) in the middle of a shed needs more time and specialized equipment. Again, spreading the heap evenly throughout the shed is also much more time consuming than building and breaking of windrow. Chicken growers prefer windrows to heaps.

A limitation of the Sydney experiment was not having any control litter treatment (untreated litter, leaving litter in the shed without heaping or windrowing). Therefore, with the consultation of industry, this experiment compared windrows (with or without turning) with no litter treatment to measure the real effect of the litter treatments (windrows) on the pathogen inactivation up to 10 days.

The main objectives of this experiment were to:

1. determine the efficacy of partial composting (windrow) in reducing litter pathogen load;
2. determine the decay profile of litter infectivity in untreated and composted litter;
3. determine the effect of windrow turning on litter composting and decay of infectivity; and
4. determine changes in temperature pH and moisture over 10 days in untreated litter, windrow composted litter with turning, and windrow composted litter without turning.

The specific hypotheses under test were:

- Pathogen load in the broiler litter will reduce over time (0–10 days).
- There will be a higher reduction of pathogen load and infectivity in the windrowed litter than untreated litter.
- Windrow turning will produce better results than the windrow alone (without turning).

Aspects of this study are reported by Islam et al. (2010a).

4.2 Materials and methods

There were two parts of the experiment, litter treatment experiment and the actual bioassay. The litter treatment experiment was conducted in a broiler farm in Brisbane (Inghams Enterprises) and the subsequent bioassay of the litter was conducted at UNE chicken isolator facilities.

The broiler farm is situated in an isolated area, three sides bounded by state forests. The nearest poultry operation is about 2 km away, although the region has reasonably dense chicken population. The farm has eight tunnel ventilated sheds. There is one recently built, extra-large shed that has a

capacity of rearing about 50,000 broiler chickens (Shed 8). All other sheds had a capacity of 35,000–40,000 broiler chickens. The experiment was conducted in sheds 1–6; two shed including the largest one were not included in the experiment (Figure 4-1).

There were three litter treatments: windrow (Win), windrow turn (WT) and no litter treatment (NLT) application. Treatments were applied to the litter of the half of each of six sheds either on the day of complete removal of chickens or a day after, whereas the litter from the other half was removed to give new litter and used as a brooding area for the next batch of chickens. The three treatments were allocated to the pre-selected six (each treatment in duplicate) out of eight sheds of the farm. The treatment allocation is shown in Table 4-1.



Figure 4-1: Aerial view of the Brisbane farm. The experiment was conducted in the bottom six sheds.

Table 4-1: Treatment allocation in the six broiler sheds of the Brisbane farm

Shed	Treatment	Shed Dimension	Windrow Dimension
1	Windrow turn (WT)	140 m × 15 m	H:80 cm, W:200 cm, L:73 m
2	No litter treatment (NLT)	140 m × 15 m	
3	Windrow (Win)	150 m × 16 m	H:100 cm, W:200 cm, L:70 m
4	Windrow turn (WT)	150 m × 16 m	H:100 cm, W:200 cm, L:76 m
5	No litter treatment (NLT)	150 m × 15 m	
6	Windrow (Win)	140 m × 15 m	H:90 cm, W:250 cm, L:65 m

Windrow (Win): All litter from half of the shed was piled in the middle of the shed longitudinally using a mechanical device. Each windrow was approximately 1.0 m (0.8 m to 1.2 m) height and 80 m long (Figure 4-2).

Windrow Turn (WT): It was exactly the same as Win, which was turned, shifted the whole windrow by a meter, so that surface litter can go underneath, using mechanical device at day 4, after collection of day 4 litter sample.

No Litter Treatment (NLT): The litter was left alone (flat) following depopulation. No piling was done (Figure 4-3). At day 4, a mechanical device was used to turn the litter and leave it flat again up to day 10, the last sampling day.

4.2.1 Physical measurements

The following physical measurements were conducted from the farm over the 10 days period.

Farm location, shed dimensions, type and orientation. Also litter depth and sampling depths from litter prior to heaping (day 0 measurement).

Temperature distribution (spatial and temporal). Continuous temperature was monitored using iButton® data loggers from various sites of the litter described below:

- *Windrow (WIN) and Windrow Turn (WT):* Temperature was recorded from 4 depths (0, 25, 50 and 75 cm) at two angles (90° and 45°) at 3 locations along the windrow (5 m from one end, middle and 5 m from the other end). The iButtons were placed in a total of 12 places in each windrow.
- *No Litter Treatment (NLT):* Temperature was recorded from 3 depths (surface, 1–2 cm depth; middle, 5–8 cm depth and bottom, 10–15 cm depth) at 8 locations around the shed (n=24/treatment replicate/sampling time).



Figure 4-2: Windrow formation and placement of iButtons® in the windrow with bamboo stakes and ribbons as markers



Figure 4-3: No Litter Treatment (NLT) sheds (litter was not windrowed).

4.2.2 Litter sample collection

Litter samples were collected at days 0, 4, 7 and 10 following the application of the treatments and transported to UNE on the same day in the evening. Samples from different depths and locations were mixed and pooled by shed so that each sampling day results in 6 samples being transported to UNE for bioassay. A portion of the same samples were also collected in 100 ml jars for moisture content and pH measurements. The procedure of sampling collection was as follows:

Day 0 (all sheds) and no treatment sheds (all sampling days). Approximately 85 ml of litter from three depths (surface, 1–2 cm depth; middle, 5–6 cm depth and bottom, 10–15 cm depth; total volume ~250 ml), was collected from 40 spots from a grid covering the whole shed and the samples were pooled by depth. After mixing and removal of 250 ml from each depth for measuring pH and moisture content, the remaining ~9 L of litter was thoroughly mixed, packed in cloth bags, surrounded with 4 L frozen chilly bricks and transported to UNE (Figure 1-11).

Day 4, 7 and 10 sampling in windrow treatments. 400 ml of litter was collected from ten sites on the surface of the each windrow (4 L), eight sites at a depth of 25 cm (3.2 L), six sites at a depth of 50 cm (2.4 L) and four sites at a depth of 75 cm (1.6 L). The litter from each depth was collected in a separate bucket and mixed thoroughly by hand. Two subsamples of 100 ml each was collected from each of these mixtures for measuring pH and moisture content ($n=3$ treatments \times 2 sheds \times 4 depths \times 2 replicates = 48 at every time point). After taking the subsamples four buckets of litter was mixed together and the total quantity (~9 L) of litter from each windrow was transported to UNE. The different volumes from each depth reflected the relative contributions to overall litter mass. A picture of the litter sample collection is presented in Figure 4-4.



Figure 4-4: Litter collection from various depths of windrows

3. Bioassay for litter infectivity

The bioassay experiment utilized a 3×4 factorial design plus two positive and one negative control group. Each treatment was replicated in two different sheds and infectivity measurements were based on a single isolator for each replicate in the field plus two replicates of positive and negative controls.

The treatment combinations in the field were:

Three litter treatment methods

- No Litter Treatment (NLT)
- Windrow (Win)
- Windrow Turn (WT, turned at day 4)

Four collection days during the litter treatment

- Day 0 post-treatment (day after destocking).
- Day 4 post-treatment (before turning the windrow in WT)
- Day 7 post-treatment
- Day 10 post-treatment

At UNE, concurrent experiment LT09-C-CB5 generated known infective litter to act as a positive control. Both this and fresh litter (pine shavings) were used as controls in the litter infectivity studies (chick bioassay). This provided the additional following treatments

Positive control (UNE litter) and

- UNE-Dir* (litter taken directly from the animal house just before putting into the isolator) – one isolator in the isolator facility and one in the animal house
- UNE-Tran* (UNE litter following a transport simulation) – one isolator in the isolator facility and one in the animal house

Negative control (fresh litter)

- One isolator in the isolator facility and one in the animal house

A total of 30 isolators were used, with 11 chickens per isolator (with the aim that we can harvest 10 serum samples from each at day 35 post exposure) providing a total of 330 SPF chickens overall and 22 chickens (2 isolators) per treatment combination. On each sampling day, 6 litter samples were collected (1 per shed) and sent to UNE for bioassay. Each litter sample was placed in a single isolator (6 sheds x 4 sampling times = 24 isolators, plus 6 additional isolators; 2 each for positive and negative controls and positive control plus simulated transport. Total = 30 isolators).

SPF chickens were purchased in two batches. Initially 198 chickens (Batch 1) were placed in 18 isolators to cover day 0 and day 4 litter treatments, plus the positive and negative controls and the transport simulation treatments. Another 132 chickens (Batch 2) were placed in the remaining 12 isolators to cover the day 7 and 10 treatments. We have previously shown that SPF chickens exposed to litter at day old or 7 days later show no difference in serological response to litter pathogens in experiment LT07-C-CB2. This design will ensure that chickens do not differ in age by more than 7 days at time of exposure to litter.

Chickens were exposed to litter by placing approximately 9 L of litter in two cat litter trays (40 x 30 cm each) in each isolator immediately after arrival of the litter at UNE (Figure 1-1). Litters remained in the trays up to three weeks, until depleted completely.

UNE-Dir and UNE-Tran litters: The positive control litter was produced by rearing 40 broiler chickens on a floor pen (4.5 m²). Approx 8–10 cm of pine wood shavings was laid on the floor. Chickens were fed broiler starter *ad lib* for the first 14 days then changed to broiler finisher. Normal brooding temperatures were used; starting at 35 °C at day 0 and declining by 1 °C every 2 days until a temperature of 21 °C was reached. Vaccination was performed as described in the Table 4-2.

Table 4-2: Vaccine and vaccination schedule of the experiment.

Vaccine	Source	Strain	Dose	Age of chickens at inoculation	Route of inoculation
MDV dust	Collected from Iso 12 of MD05-R-PT3	MDV dust	Spread on litter	12 days	Spread on litter
Vaxsafe IBD	Bioproperties	Strain V877	>10 ^{2.4} EID50/dose	14 days	Eye drop
Steggles CAV vaccine	Dr Tom Grimes	Strain 3711	10 ^{1.575} CID50/dose	16 days	Oral, x10 dose used
Vaxsafe ND	Bioproperties	Strain ND V4	>10 ^{6.0} EID50/dose	18 days	Eye drop
Vaxsafe IB	Bioproperties	IBV Ingham's strain	10 ^{3.0} EID50/dose	19 days	Eye drop

At day 27 of age, 10 chickens were bled and all chickens were removed. Sixteen litres of litter was stored in an esky with 4 L of ice and then placed in a climate control chamber where temperature was maintained from 16–35 °C as mentioned in the Chapter 2 (Figure 2-1). This litter is termed as *UNE-Tran* and used in the subsequent bioassay. Chickens of two isolators were exposed to this litter on the next day with all other litter from the field experiment. On the infection day, another 16 L of litter was collected from this broiler chickens and placed into two isolators directly and this litter is called as *UNE-Dir*.

The shedder chickens were serologically positive for MDV (100%, 9/9), IBV (100%, 9/9), IBDV (78%, 7/9) and CAV (11%, 1/9).

Measurements during the bioassay: All mortalities were examined for gross pathology on post-mortem.

At day 35 post exposure to litter, blood samples were collected. There were four different bleeding days for four days of exposure. At day 42 days of age, chickens were humanely killed, body weight recorded before disposing.

Serum samples were analysed for serology for IBV (ELISA), CAV (ELISA), IBDV (ELISA) and NDV (ELISA) and FAV8 (ELISA), ILTV (ELISA) and MDV at UNE. Commercial kits were used ELISA from IDEXX Laboratories Inc (Maine, USA) for IBV, CAV, IBDV and NDV. Kits from TropBio Pty Limited (JCU, Townsville, QLD) was used for FAV/IBH and ILT. MDV ELISA was developed at UNE using vaccinal MDV1 virus (Rispens CVI988).

Litter pH measurement: pH was measured using a pH meter. Two and a half grams of litter samples were weighed in a screw cap plastic jar, where 50 ml of Milli-Q water was added. The content was shaken vigorously for one minute to mix thoroughly. The mixture was kept at room temperature for 30 min. Within this waiting period, the contents were shaken vigorously every five minutes. The pH meter was calibrated using standard buffers of pH 4 and 7 before reading the sample. The electrode was thoroughly rinsed in between sample reading. The instrument was calibrated with pH 7 buffer after every 4 samples.

Dry matter measurement: Litter dry matter was determined after drying about 50 g of litter at 80 °C for 48 hours.

Litter chemistry: Dried litter samples (80 °C) were ground with a fine a grinder. The ground litter was sieved through a 0.2 mm sieve and collect then sieved again through a 0.05 mm sieve and collect separately. The 0.2 mm sieved products were used for determining potassium (K), sodium (Na), phosphorus (P) and other trace elements using an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES, Carlo Erba Instruments, Strada Rivoltana, Milan, Italy) and the 0.05 mm products were used to determine carbon and nitrogen content using Carlo Erba NA 1500 Solid Sample Analyser as described previously.

Statistical analysis. Analyses were performed with JMP7 (SAS Institute Inc. 2007). Where the chicken was the experimental unit measured discrete data (serologically positives and negatives are coded as 1 and 0 respectively), analyses was done using a generalized linear model with a binomial link function (logistic) fitting the effects of treatment, day and their interaction. All continuous data are analysed using general linear model fitting the effects treatment, depth (if appropriate), day and their 1st order interactions. Least squares means and standard errors are presented.

A specific description of the analytical model is given in each subsection of the results section.

4.3 Results

4.3.1 Litter temperature

Litter temperature was recorded up to 10 days from four depths (surface, 25, 50 and 75 cm) for windrow (Win) and windrow turn (WT) and at three depths (surface, middle and bottom) in the no litter treatment (NLT).

The temperature profiles over time of each treatment are presented in Figure 4-5. As expected, the temperature was much higher in Win and WT than NLT. There was not much variation in the temperature profiles of Win and WT (Figure 4-5). When compared within each treatment, highest temperature was recorded at the bottom of the litter followed by middle and surface in NLT (Figure 4-6). The temperature inside the NLT was much more consistent and maintained around 30 °C whereas surface temperature of the NLT was much lower and more variable (depending on the time of the day).

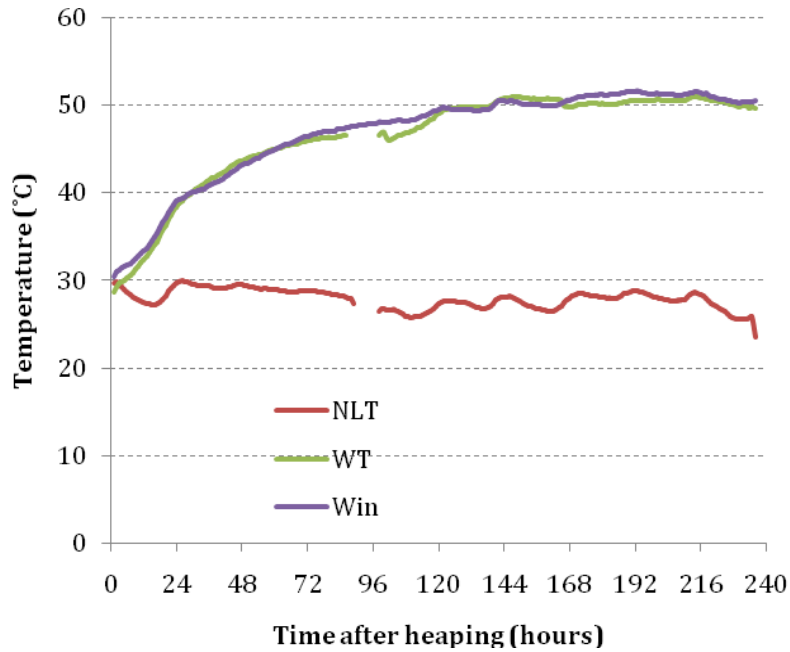


Figure 4-5: Temperature profile (mean of 24 readings in each curve) in Windrow (Win), Windrow Turn (WT) and No Litter Treatment (NLT) group over time (all depths included). The missing of data in the curves of the WT and NLT at around 90–98 hour was due to taking off the temperature probes during turning the litter.

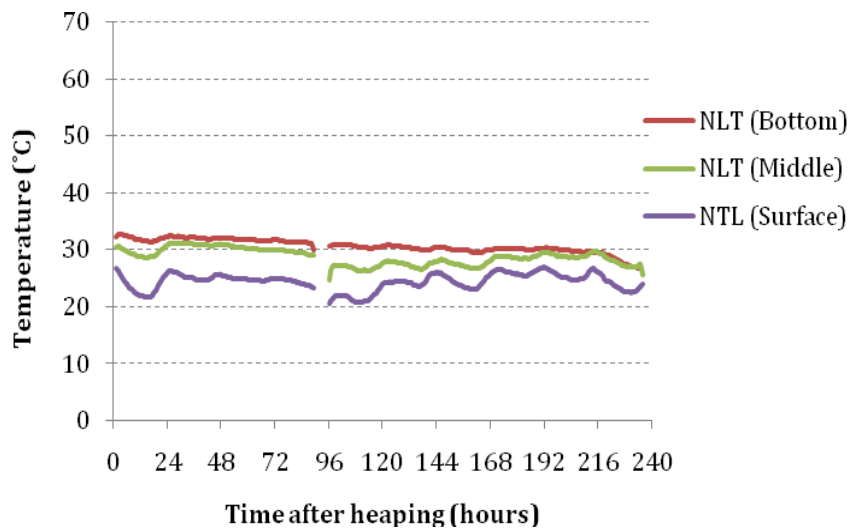


Figure 4-6: Temperature profile (mean of six readings in each curve) in No Litter Treatment (NLT) group at three heights over time. The missing of data in the curves at around 90–98 hour were due to taking off the temperature probes during turning the litter.

In Win and WT, overall, the temperature at 25 cm depth went up quickly followed by 50 cm depth and 100 cm up to four days (96 hours). However, after day 4, the temperature of 50 cm went up and maintained well over 60 °C. The temperature at 25 cm remained relatively steady (under 60 °C) and went down at the final hours of the experiment (day 10). The temperature at 75 cm was always well below the temperatures of 25 and 50 cm, but increasing consistently up to day 10 (Figure 4-7).

The temperature of various depths at WT and Win shows identical patterns as presented in Figure 4-7 and Figure 4-8.

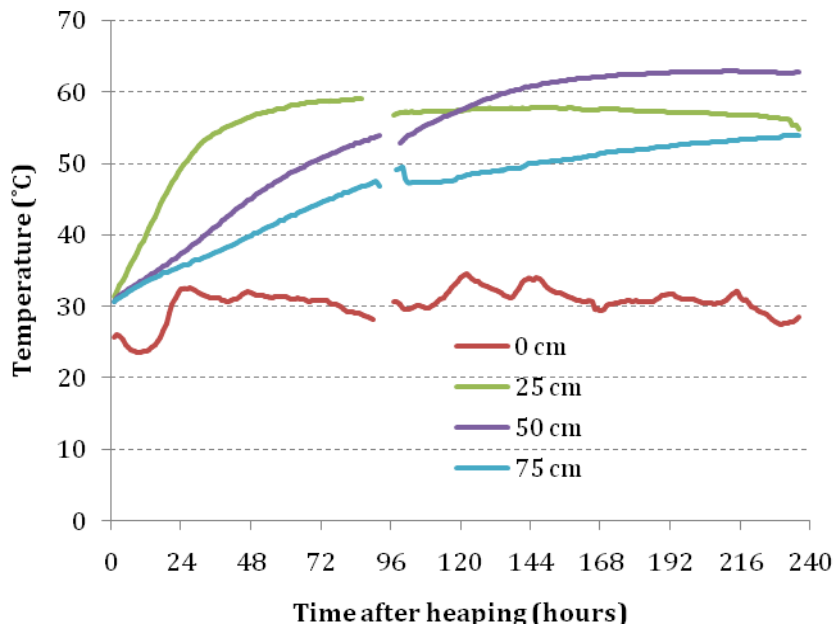


Figure 4-7: Temperature profile (mean of 18 readings for each curve) at various depths for Win and WT. NLT was not included because it has different depths. The missing data in WT and NLT at around 90–98 hour was due to taking off the temperature probes during turning the litter.

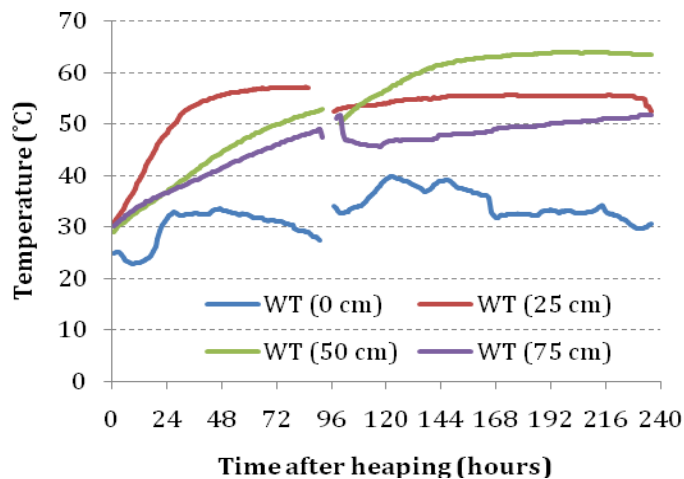


Figure 4-8: Temperature profile (mean of six readings in each curve) in WT group at four heights over time. The missing of data in the curves at around 90–98 hour were due to taking off the temperature probes during turning the litter.

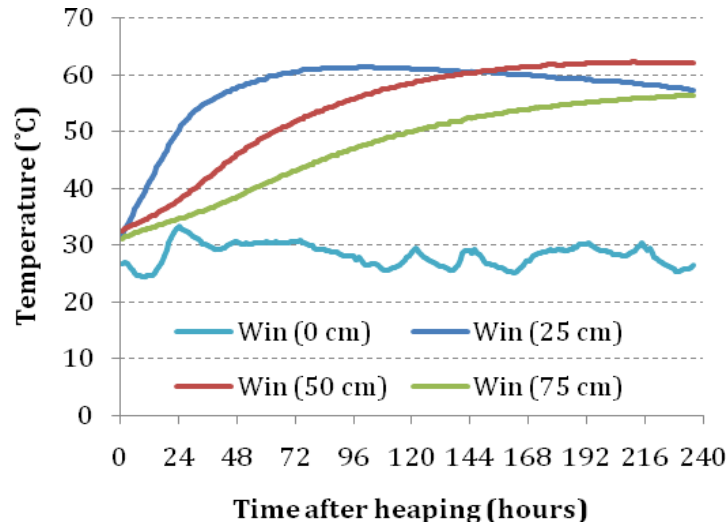


Figure 4-9: Temperature profile (mean of six readings in each curve) in the windrowed (Win) group at four heights over time.

To compare the mean temperature between treatments, two separate analyses were conducted. In the first analysis, comparison was done up to 90 hours, before taking off the iButtons for tuning, to avoid the drop of temperature during turning. The second analysis was a comparison of temperature after the turning (96 hours onward).

Before litter turn (day 0 to 4), there was a significant effect of treatment ($P < 0.001$) on the litter temperature with a lower temperature in NLT (28.7 °C) group than Win (41.4 °C) and WT (41.0 °C), however there was no significant difference in temperature between Win and WT litter. Within Win and WT groups, temperature was lower on the surface (29.2 °C), than temperature inside the litter. Overall, the highest temperature was achieved at 25 cm (52.2 °C) depth followed by 50 (43.5 °C) and 75 cm (39.5 °C) depths (Figure 4-10, left). The depth had an effect on the temperature within NLT treatment too.

After the litter turn (day 4 to 10), there was a significant effect of treatment with the lowest temperature in NLT (27.3 °C) followed by WT (49.7 °C) and Win (50.2 °C). Again, within Win and WT, the temperature was lowest on the surface (31.1 °C). The highest temperature was found at the depth of 50 cm (60.7 °C) followed by 25 cm (57.3 °C) and 75 cm (50.9 °C) (Figure 4-10, right).

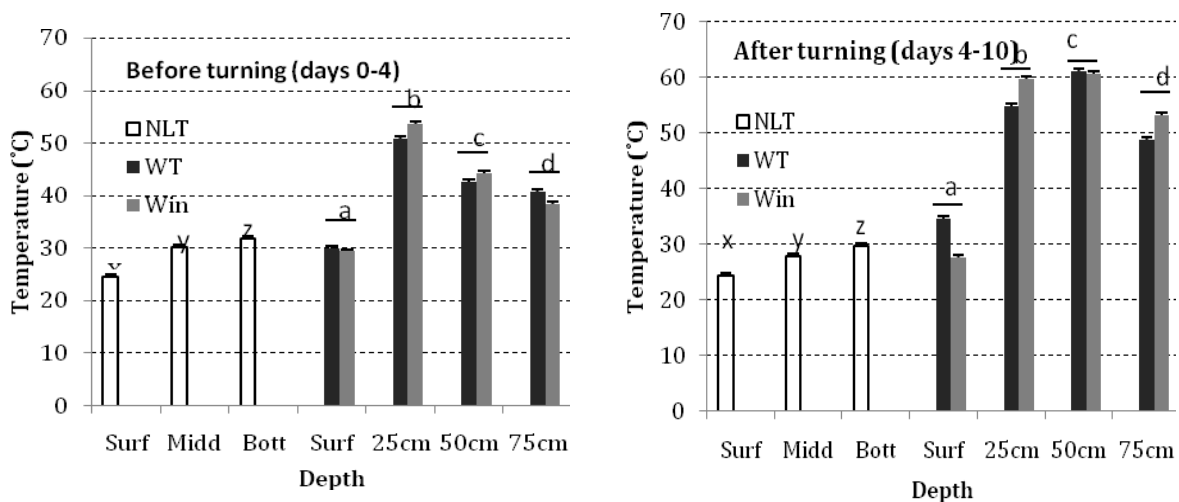


Figure 4-10: Mean (LSM±SEM) temperature at various depths in all three treatments before (left) and after (right) turning the litter at day 4. Column not sharing a common letter are significantly different from each other within the same series.

4.3.2 Litter pH

As mentioned in the design of the experiment, litter samples were collected from three depths (surface, middle and bottom) at day 0 and NLT for all days. For Win and WT, litter samples were collected from four depths at days 4, 7 and 10. The day 0 samples were collected before applying any treatment. In other words, the design of the litter study was not full factorial. Therefore full model analysis was not possible. The first analysis of the full data set was conducted to test the main effect of day and treatment. A second analysis was conducted to explore the effect of depth and treatment excluding all day 0 data from Win and WT and all data from NLT. The third analysis was conducted to test the effect of depth on all day 0 data. The effect depth and day was explored finally within NLT.

Analysis of all data, considering two factors (day and treatment) revealed a significant effect of day ($P < 0.001$) but not treatment ($P = 0.39$) on the litter pH with a significant interaction between the main effects ($M < 0.01$). Litter pH was decreasing over time until day 10 in Win and WT but not in NLT, where it was decreased up to day 7 and then increased at day 10 (Figure 4-11).

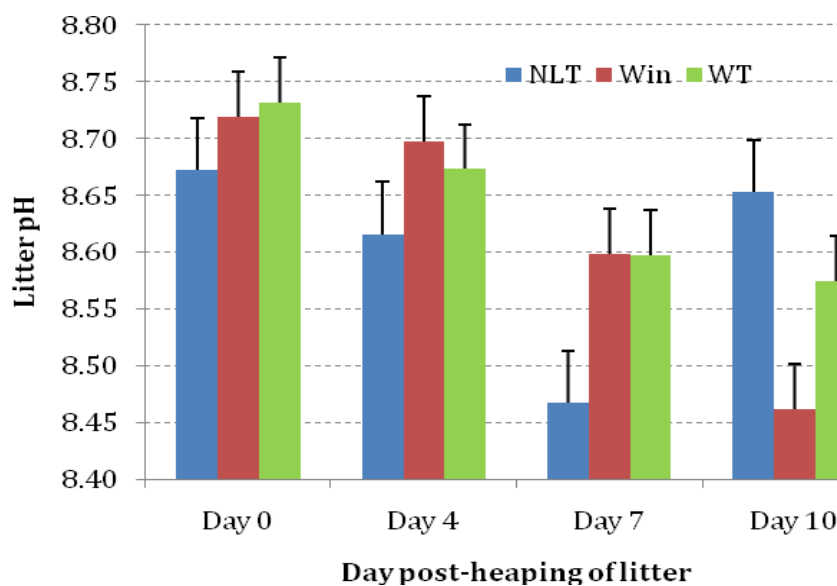


Figure 4-11: Mean (LSM±SEM) litter pH at various days post-treatment in Windrow (Win), Windrow turn (WT) and NLT.

Analysis within Win and WT showed no significant effect of treatment ($P = 0.35$) and depth ($P = 0.20$) with no significant interaction ($P = 0.19$) between the main effects (Figure 4-12, *left*). There was a significant effect of depth was found when only day 0 data were analysed separately with a lower pH in the bottom than middle and surface litter (Figure 4-12, *right*).

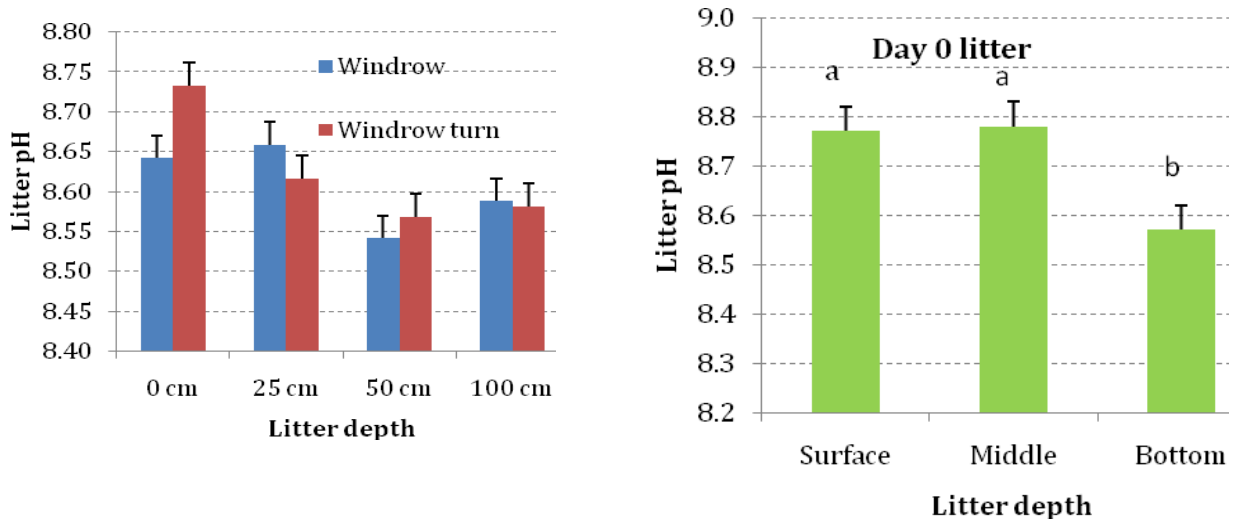


Figure 4-12: Mean (LSM±SEM) litter pH at different depths of windrowed (Win) and windrowed turned (WT) litter (left). Litter pH (LSM±SEM) of day 0 in various depths (right).

When the NLT treatment was analysed for the effect of day and depth, there was significant effects of day ($P<0.001$) and depth ($P<0.001$) with their interaction ($P<0.001$). The pH of the surface (8.4) litter was much lower than middle (8.7) and bottom (8.7) litter. The day 7 litter pH was lower than any other days. Significant interaction was represented by much lower pH at day 7 from the surface litter (Figure 4-13).

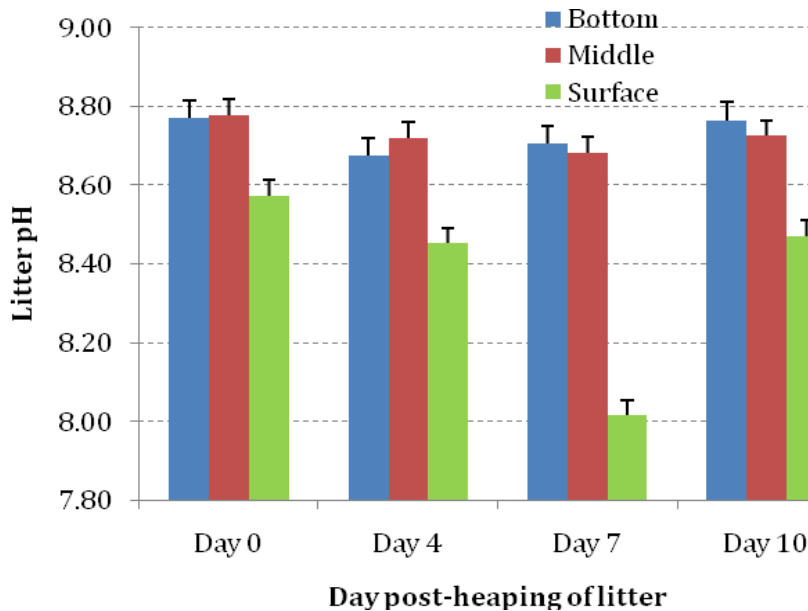


Figure 4-13: Mean (LSM±SEM) litter pH at various days post treatment in the no litter treatment (NLT) group (Win and WT were excluded).

4.3.3 Litter dry matter

Analyses were conducted as with litter pH.

There was a significant effect of day ($P<0.02$) but not treatment ($P=0.67$) on the litter dry matter with a significant interaction between the main effects ($P<0.05$). Not much change in the dry matter observed with day except it was lower at day 7 than days 0 and 4 but not from day 10 (Figure 4-14).

When analysed Win and WT data, there was no significant effect of depth ($P=0.35$) and treatment ($P=0.37$) on the litter dry matter, interaction between the main effects was also non-significant ($P=0.97$) (Figure 4-15, *left*). There was a significant effect of depth on the litter DM within day 0 data with a lower DM in bottom than middle and surface litter (Figure 4-15, *right*). There was no significant effect of Depth on the DM content of litter within NLT treatment.

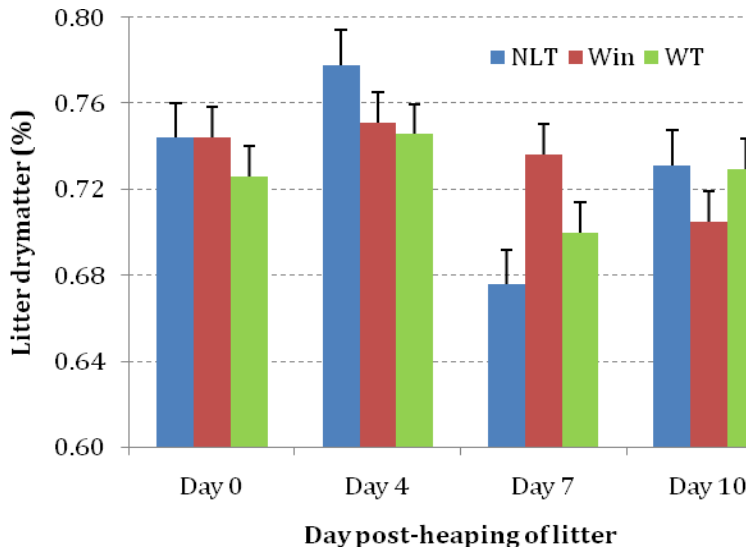


Figure 4-14: Mean (LSM±SEM) litter dry matter at various days post-treatment in windrow (Win), windrow turn (WT) and no treatment litters (NLT).

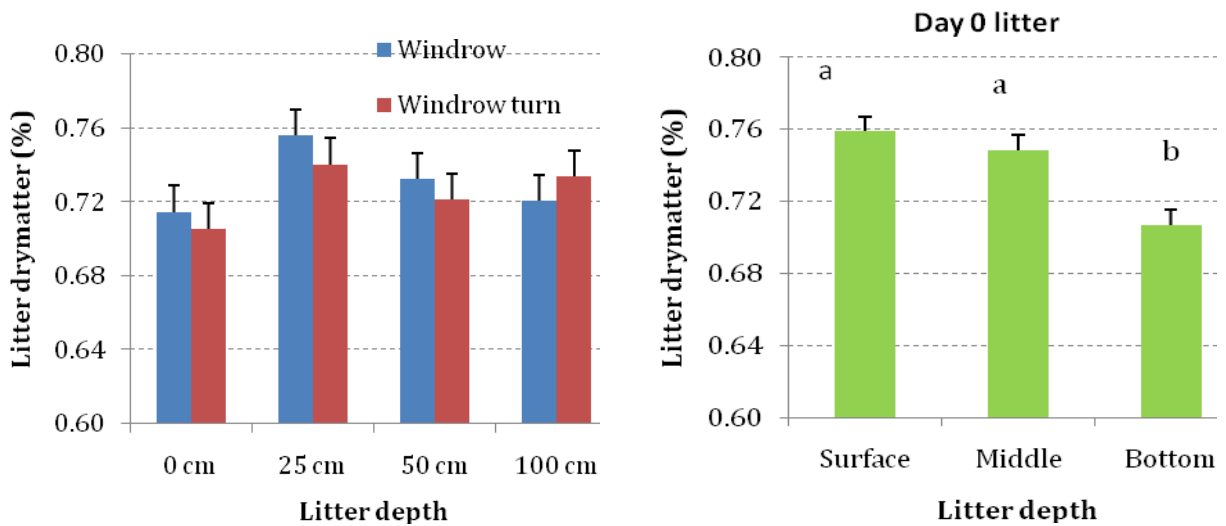


Figure 4-15: Mean (LSM±SEM) litter dry matter at different depths of windrow, windrow turn (left panel) and no treatment (right panel) litters.

4.3.4 Association between litter pH and dry matter

There was a significant positive association between the litter dry matter in pH ($P<0.001$, $R^2=0.37$), the higher the dry matter the higher the pH (Figure 4-16).

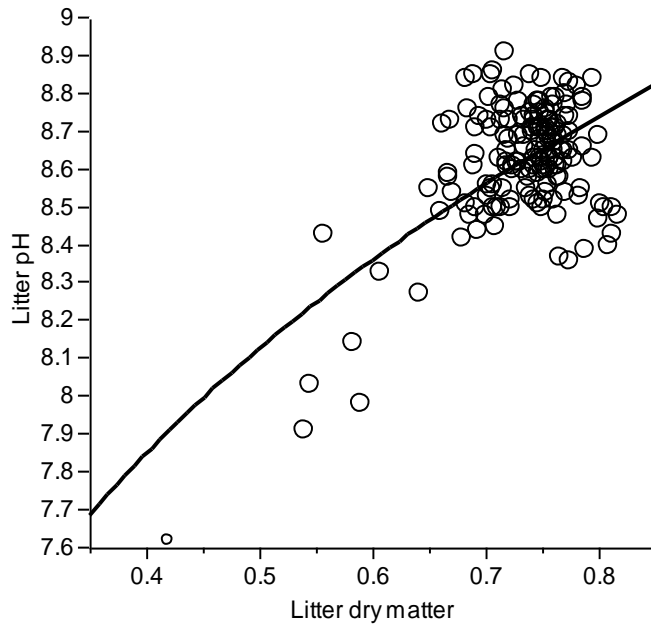


Figure 4-16: Association between litter dry matter and pH.

4.3.5 Bioassay for litter infectivity

4.3.5.1 MDV serology

All serology data were coded as 0 and 1 for negative and positive respectively (response) and analysed using generalized linear model (GLM) of binomial variable. The model tested the effect of day and treatment. As the negative control, *UNE-Dir* and *UNE-Tran* litters had only one day, those data were excluded during factorial analysis. However those groups were included in a separate analysis for treatment comparison.

There was a significant effect of Day ($P < 0.001$) on the MDV response but not Treatment ($P = 0.60$) and their interaction ($P = 0.45$). Contrast analysis showed that the MDV incidence was significantly lower in day 10 litter than any other days ($P < 0.001$), however, there was no significant differences in the incidence of MDV between days 0 and 7 ($P = 0.06$), days 0 and 4 ($P = 0.30$) days 4 and 7 ($P = 0.30$). This indicates that the MDV decay in the litter does not depend on the litter treatment; time is the only factor inactivating MDV in the litter.

Analysis including all data showed that there was no significant difference in MDV response in any treatment (including *UNE-Dir* and *UNE-Tran*) group except the negative control group. There were 3/20 negative control chickens were positive (very low titre) for MDV, which was unexpected. These might represent false positive, however, at this stage we are not certain about that. The percentage of MDV response data are presented in Figure 4-17.

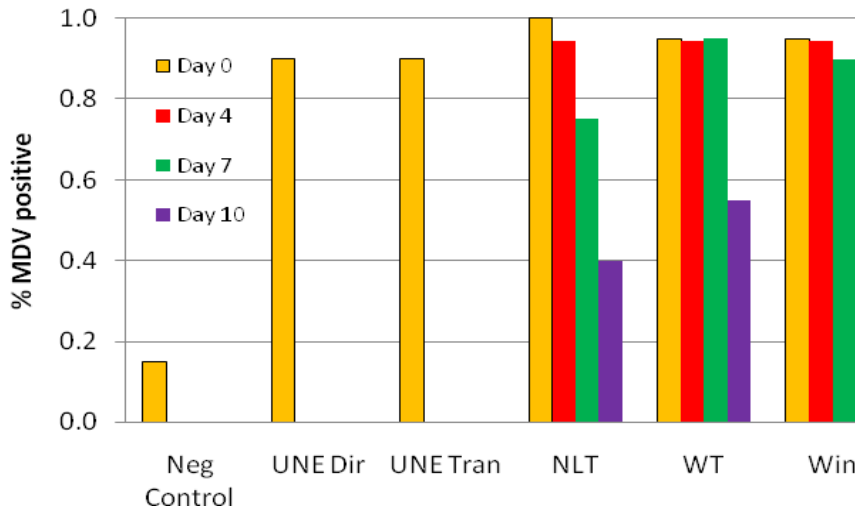


Figure 4-17: Percentage of chickens serologically positive for MDV in various treatment groups. Treatment by day interaction.

Analysis of MDV ELISA titre ($\log_{10} + 1$) revealed similar results to MDV response. Analysis of the full data set revealed a significant effect of treatment on the MDV titre, having significantly lower titre in the negative control group than all other groups, which are not different from each other (Figure 4-18).

Full model analysis of the data excluding negative control and UNE litter data showed a significant effect of day ($P < 0.0001$) but not treatment ($P = 0.24$) and there was significant interaction ($P < 0.26$) between two main effects (Figure 4-19). The mean MDV titre was significantly lower in day 10 than other days, however there was no difference in the titres of other days (Figure 4-18).

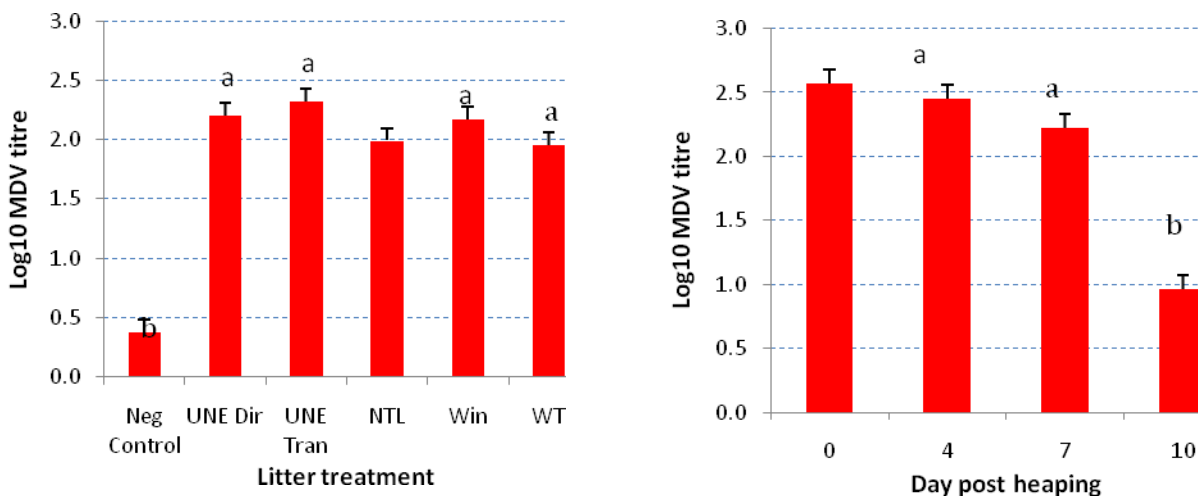


Figure 4-18: MDV titres (LSM of $\log_{10} + 1 \pm \text{SEM}$) in various treatment groups. Main effects of treatment and day.

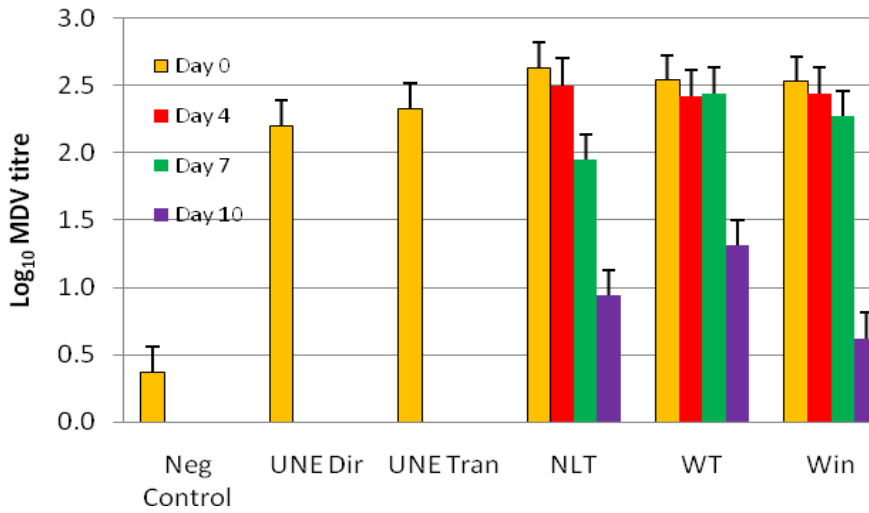


Figure 4-19: MDV titres (LSM of Log₁₀ + 1±SEM) in various treatment groups. Treatment by day interaction.

4.3.5.2 FAV/IBH serology

Analysis using GLM of binomial data revealed that significant effects of treatment (P<0.001) and day (P<0.001) on the FAV response with a significant interaction (P<0.001) between the main effects. The FAV response was significantly (P<0.0006) higher in NLT group (71%) than Win (44%) or WT (36%), however there was no significant (P=0.33) difference between the later two treatments (Table Table 4-3). The FAV response was going down with increasing duration of litter treatments up day 7 in all treatments. The rate of decrease was much faster in Win and WT than NLT group; however, there was an increase in the FAV response at day 10 in the Win litter (Figure 4-20).

There was no chicken was positive for FAV in *UNE-Dir* and *UNE-Tran* group.

Table 4-3: Percentage of chickens serologically positive for IBH/FAV in litter following heaping or windrow up to 10 days

Treatment	Day	Positive	Negative	%
Control		0	17	0
Win	0	15	5	75
	4	8	10	44
	7	0	20	0
	10	11	20	55
WT	0	19	1	95
	4	9	9	50
	7	0	20	0
	10	0	20	0
NLT	0	19	20	95
	4	17	1	94
	7	10	10	50
	10	9	11	20
<i>UNE-Dir</i>		0	18	0
<i>UNE-Tran</i>		0	20	0

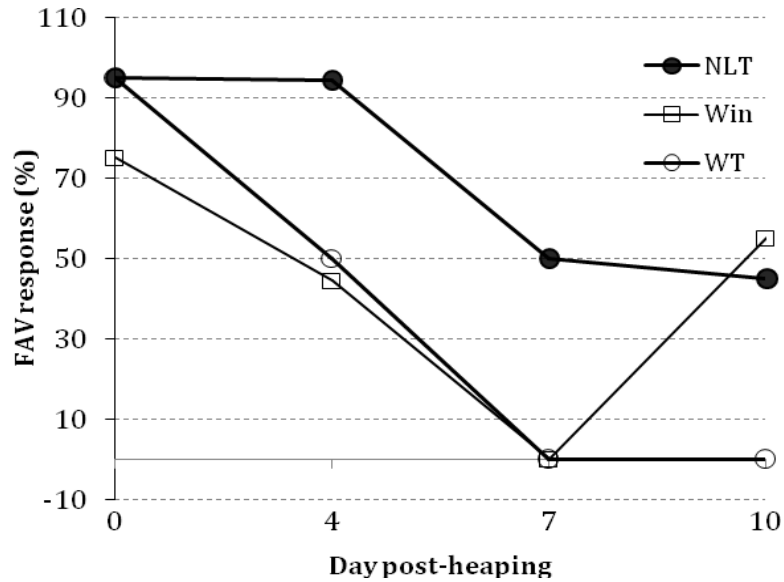


Figure 4-20: Percentage of chickens serologically positive for FAV at various days post treatment of litter.

There was a significant effect of day ($P < 0.001$), treatment ($P < 0.001$) and their interaction ($P < 0.001$) on the \log_{10} FAV titre. Titre decreased to zero by day 7 in Win and WT group but a less reduction was recorded in NLT group. However, the WT maintained its zero level up to day 10 but there was an increase of titre at day 10 in Win. There was no reduction of titre in NLT group from day 7 to day 10 (Figure 4-21).

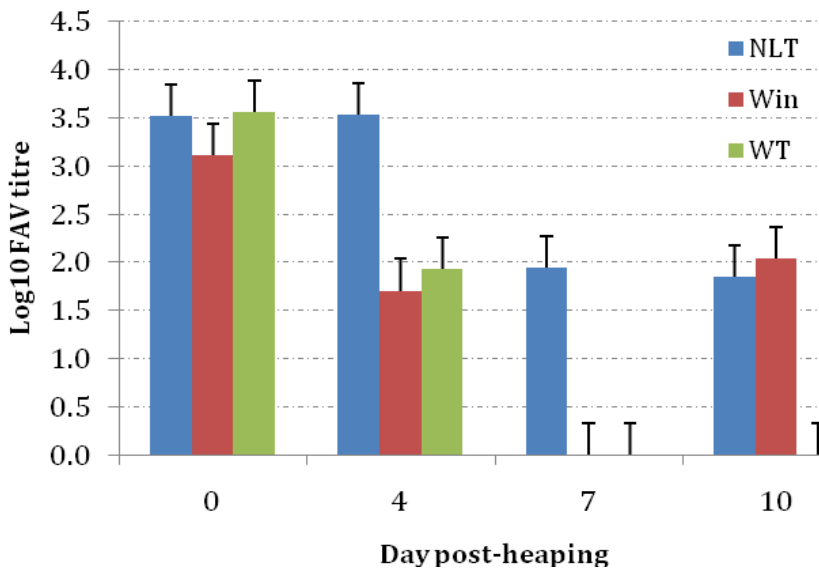


Figure 4-21: FAV/IBH titres (LSM of $\log_{10}+1 \pm \text{SEM}$) at various days post-treatment of litter

CAV serology

There was a significant effect of day ($P < 0.001$) and treatment ($P < 0.001$) but not their interaction ($P = 0.16$) on the CAV response. The CAV response was significantly higher at day 0 than any other days including day 4 ($P < 0.001$). There was a significant difference in the response between day 4 and 7 ($P < 0.02$) but not between day 7 and 10 ($P = 0.99$). Overall, there was a significantly higher CAV response in NLT (53%) than Win (12%) and WT (26%) ($P < 0.001$). The response was significantly higher in Win than NLT ($P < 0.02$) (Table 4-4, Figure 4-22). These data suggest that decay of CAV in

litter does not depend on the time alone, litter treatments such as windrowing can enhance the decay of the virus over time. However, after 10 days, significant infectivity was retained.

Chi-square analysis showed that there was a higher CAV incidence in *UNE-Dir* than *UNE-Tran* litter ($P < 0.01$).

Table 4-4: Percentage of chickens serologically positive for CAV in litter following heaping or windrow up to 10 days

Treatment	Day	Positive	Negative	%
Control		0	17	0
Win	0	13	7	65
	4	1	17	6
	7	0	20	0
	10	0	20	0
WT	0	19	1	95
	4	3	15	17
	7	1	20	5
	10	1	20	5
NLT	0	19	20	95
	4	15	3	83
	7	8	12	40
	10	7	13	35
<i>UNE-Dir</i>		9	11	45
<i>UNE-Tran</i>		2	18	10

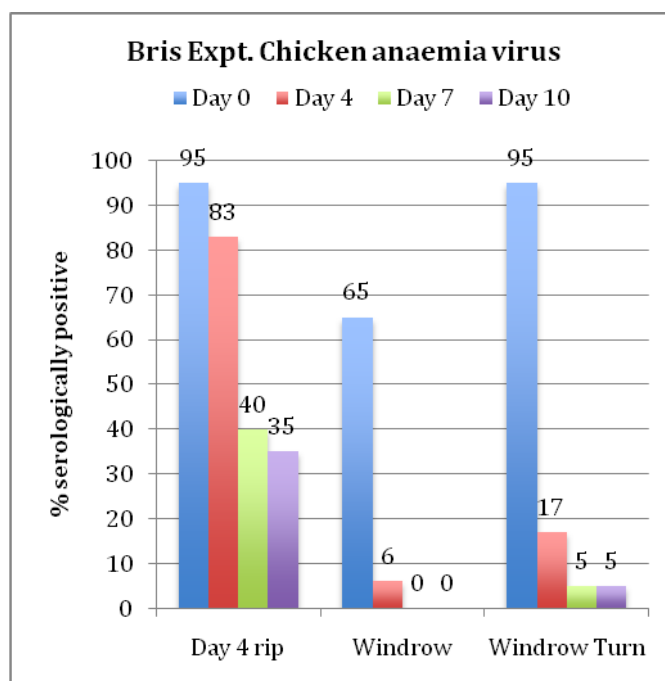


Figure 4-22: Percentage of chickens serologically positive for CAV at day 35 post exposure to litter. Interaction plot of effects of treatment and day of litter collection post heaping.

IBDV serology

There were only a few chickens was positive for IBDV (Table 4-5, Figure 4-23). Statistical analysis revealed no effect of any factor (day or treatment). There was no difference in the response in *UNE-Dir* and *UNE-Tran* litters, as all chickens in these two groups were positive.

Table 4-5: Percentage of chickens serologically positive for IBDV in litter following heaping or windrow up to 10 days

Treatment	Day	Positive	Negative	%
Control		0	17	0
Win	0	1	19	5
	4	0	17	0
	7	0	20	0
	10	0	20	0
WT	0	5	15	25
	4	3	15	17
	7	0	20	0
	10	0	20	0
NLT	0	0	20	0
	4	0	3	0
	7	0	12	0
	10	0	13	0
<i>UNE-Dir</i>		20	0	100
<i>UNE-Tran</i>		20	0	100

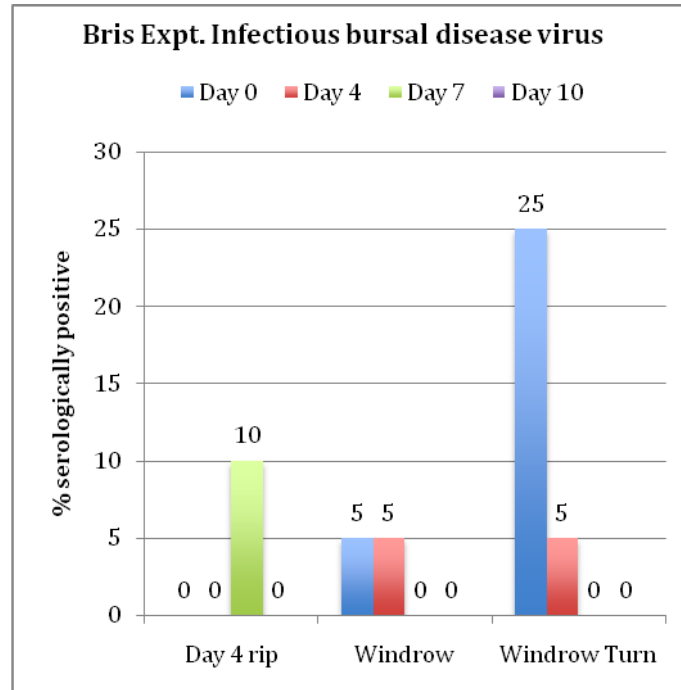


Figure 4-23: Percentage of chickens serologically positive for IBDV at day 35 post exposure to litter. Interaction plot of effects of treatment and day of litter collection post heaping.

4.3.5.3 IBV serology

Only a small number of chickens were positive of IBV (Table 4-6), so no analysis was applied to this data set.

Table 4-6: Survival of IBV in litter following heaping or windrow up to 10 days

Treatment	Day	Positive	Negative	%
Control		0	17	0
Win	0	1	19	5
	4	0	20	0
	7	0	20	0
	10	0	20	0
WT	0	0	20	0
	4	0	20	0
	7	2	18	10
	10	0	20	0
NLT	0	0	20	0
	4	0	20	0
	7	2	18	10
	10	0	20	0
<i>UNE-Dir</i>		0	20	0
<i>UNE-Tran</i>		0	20	0

4.3.5.4 NDV serology

No chicken was positive for NDV.

4.3.6 Chicken mortality

Only four chickens died or were euthanised during the whole experimental period without any significant effect of Treatment (4/330 = 1.2%). One bird was at day 10 with no visible lesions (NVL) as was another bird on day 31. On day 46 two chickens were autopsied, both being severely stunted in growth. One had no distinctive lesions while the other had extensive peri-hepatitis, pericarditis and peritonitis.

4.3.7 Body weight

There was a significant effect of day ($P < 0.04$) and sex ($P < 0.001$) but not treatment ($P < 0.06$) on the body weight (BW) of chickens with a higher BW in chickens exposed to *UNE-Dir* (656±14 g) and day 7 (637±10 g) and 10 (631±11 g) than day 0 (593±10 g) litters. There was also a higher BW in male (569±8 g) than females (569±7 g). There was no significant difference in the BW of chickens exposed to day 4 (596±11 g) litter with litter of other days (Figure 4-24). There was no significant difference between the BW of *UNE-Dir* and *UNE-Tran* chickens.

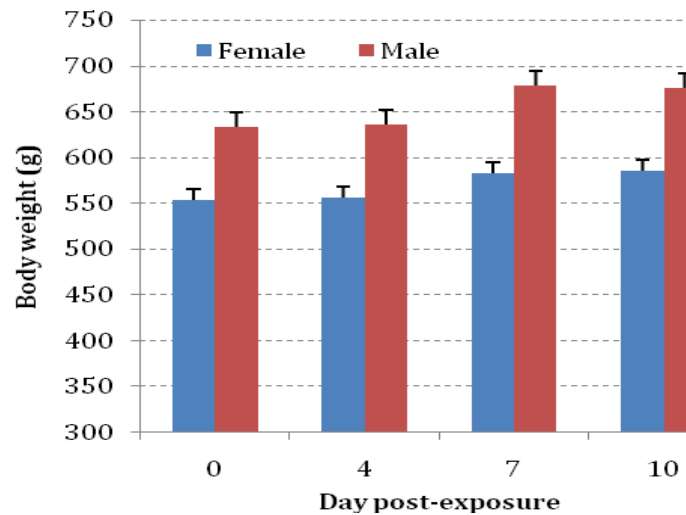


Figure 4-24: Body weight (LSM of $\log_{10}+1 \pm \text{SEM}$) of chickens exposed to litter at various days post treatment. All on farm litter treatments included. Interaction plot for effects of sex and day.

4.3.8 Summary and discussion

With regards the original hypotheses our findings were as follows:

Hypothesis 1. Pathogen load in the broiler litter will reduce over time (0–10 days). This was certainly true for CAV, MDV and FAV but not IDBV for which there were low numbers of positives overall.

Hypothesis 2. There will be a higher reduction in the windrowed litter than untreated litter. This was true for CAV and FAV, but not MDV. This suggests that declines in CAV and FAV infectivity are temperature dependant, whereas those of MDV are not, within the range of temperatures achieved during the experiment.

Hypothesis 3. Windrow turning will produce better results than the windrow alone (without turning). There was no statistical support for this hypothesis. While there was a trend towards improved inactivation of FAV in turned windrows there was an opposing trend towards reduced inactivation of CAV in turned windrows. The lack of a difference is not very surprising as the temperature profiles for Win and WT treatments were almost identical (Figure 4-5). Nevertheless the WT treatment could have been expected to expose the cool surface litter to inactivating temperatures following turning at day 4 (Figure 4-7 to Figure 4-9)

Other comments and conclusions. The temperatures from 25 cm to 75 cm depths in the windrow reached 40–50 °C by day 4 and continued to increase thereafter in different trajectories. In the Windrow treatment, at 75 cm (deepest) temperatures continued to increase slowly right up to day 10 when a temperature of approximately 56 °C was achieved. At 50 cm temperatures increased more rapidly, reaching 60 °C on day 6 and continuing to increase slowly to 62 °C by day 10. At 25 cm depth temperature increased rapidly to 60 °C by day 4, held this temperature to around day 7, then declined slowly to around 57 °C by day 10. However, the temperature on the surface of the litter fluctuated around 25–30 °C.

The temperatures measured in the windrows in this experiment were slightly lower overall than those measured in the Sydney experiment (Chapter 3) in which mean temperatures for all depths combined (including surface) peaked at approximately 55 °C at about day 3 (Compare figures 2.4 and 3.6). In the present experiment, mean temperatures of 50 °C were not reached until day 5 and they were then sustained at this level until day 10. This difference is likely to be due to the taller and wider windrows used in the Sydney experiment. The initial dry matter (approximately 74% in both experiments) or pH (approximately 8.7 in both experiments) did not differ between the two experiments.

There were not any drastic changes in the litter chemistry (pH, dry matter) due heaping or windrowing over time up to 10 days. There was a significant positive association between litter dry matter content

and pH such that wetter litters were more acidic (Figure 3-15). This confirms the same finding seen in Chapter 3.

The significantly higher bodyweight of chickens exposed to litter 7 and 10 days post treatment compared to those exposed to fresh litter (day 0) is consistent with the result observed in Chapter 3. It is probably that the high load of pathogens in fresh day 0 litter contributed for the lower BW in these chickens.

In conclusion this experiment has shown:

Windrows are capable of generating temperatures of 60 °C or higher in their core, but the temperature profile varies greatly with depth within the windrow.

Litter left on the ground but merely turned at day 4 does not generate significant temperatures.

There was a significant reduction of CAV, MDV and FAV infectivity in litter over the 10 day treatment period as determined by the chick bioassay. For CAV and FAV this decline was dependant on windrowing the litter (probably a temperature effect), but for MDV the effect was present even in the unheaped litter.

Turning of windrows did not improve the level of viral inactivation detected in this experiment.

The results of this experiment in terms of both litter measurements and effects of heaping/windrowing on viral inactivation are in very close agreement with those of the Sydney experiment reported in Chapter 3.

These data provide ongoing support for the concept of the chick bioassay as an effective means of testing loss of infectivity of pathogens in litter during litter treatments.

5 Spatial and temporal distribution of ammonia and dust concentrations in broiler sheds

Experiment 7-LT07-C-FS1

Start: 26/04/09

Completion: 04/03/09

5.1 Introduction

Ammonia (NH₃) is a colourless irritant alkaline gas produced during the decomposition of organic matter by bacterial de-amination of nitrogenous substances in the poultry shed. High NH₃ is detrimental to chicken welfare and productivity, causing reduced feed intake and growth and increased susceptibility to pathogens (Becker et al., 2002; Miles et al., 2004; Ritz et al., 2004). The reported concentrations at which adverse affects occur vary but an exposure limit of 25ppm has generally been set by industry and welfare bodies of different countries on human safety and animal welfare grounds (Kristensen and Wathes, 2000; Wathes et al., 1983). Ammonia concentrations in broiler sheds depend on many factors including litter moisture level, pH, ventilation rate and chicken age. In-shed NH₃ could also vary depending on location within a shed and this effect may differ between conventional and tunnel ventilated sheds.

The principal aim of this project is to optimize litter reuse in the broiler industry. There are reports that reuse of litter can increase shed ammonia levels very early in the chicken batch (Miles et al., 2004). The dust level could also be expected to increase in sheds using reused litter. To investigate these issues we planned to conduct a series of longitudinal field studies comparing ammonia concentrations in broiler sheds using new and reused litter at various stages of chicken's age. However, to monitor these variables over long periods, the issue of where to locate the monitoring instrumentation within the shed becomes important and this may vary between tunnel ventilated or conventional sheds. There is little or no published data on the spatial variability of ammonia and dust concentrations within a poultry shed to assist with this decision.

This study was therefore conducted to determine the spatial and temporal variation of NH₃ and dust in both types of broiler sheds at various chicken ages to resolve this issue.

5.2 Materials and Methods

The experiment was conducted at eight co-operating Cordina Poultry Farms broiler farms in Mangrove Mountain and north-western Sydney basin areas between 26 March and 4 April 2009 and details are given in Table 5-1.

Table 5-1: Details of the experimental sheds and chickens.

Sample date	Shed type	Shed size (m)	Ventilation system	Chicken age (days)	Sex	Number of birds
27/03	Conv.	70 × 14	Blinds down	7	Mixed	21,000
28/03	Tunnel	160 × 14	1 x low speed fan	6	Male	40,000
30/03	Conv.	120 × 16	Blinds down	14	Mixed	29,400
31/03	Tunnel	100 × 14	Fans on auto	22	Mixed	21,800
01/04	Tunnel	120 × 15	Fans on auto	33	Mixed	29,500
02/04	Conv.	100 × 13	Completely open	34	Mixed	17,600
03/04	Tunnel	138 × 16	Fans on auto	45	Male	17,000
04/04	Conv.	112 × 13	Completely open	46	Mixed	22,000

Aerial ammonia concentrations were measured (in ppm) using VRAE7800 Hand Held Gas Surveyors (Geotech Environmental Equipment, Inc., Colorado, USA) calibrated against a 50 ppm NH₃ standard sample. Wind speed, humidity and temperature were recorded with Kestrel Weather Meter K4000 (Nielsen-Kellerman, Inc., PA, USA). All equipment had a data logging function. Both of these instruments are shown in Figure 5-1.

Particulate matter in the shed air was measured (using units mg/m³ air) using DustTrak™ Model 8520 aerosol monitor (TSI Inc, Minnesota, USA). The machine measures dust particles ranging from 0.1 to 10 µm using light scattering technology with a laser photometer (Figure 5-1). This range of dust particle sizes includes the **respirable** dust fraction comprising particles approximately 5 µm and less, which penetrate into the gas exchange region of the lungs, and is therefore the most hazardous particulate size. It also includes part of the total **inhalable** dust fraction that is the fraction of airborne particles which enters the nose and mouth during normal breathing and is made up of particles of 100 µm diameter and less.

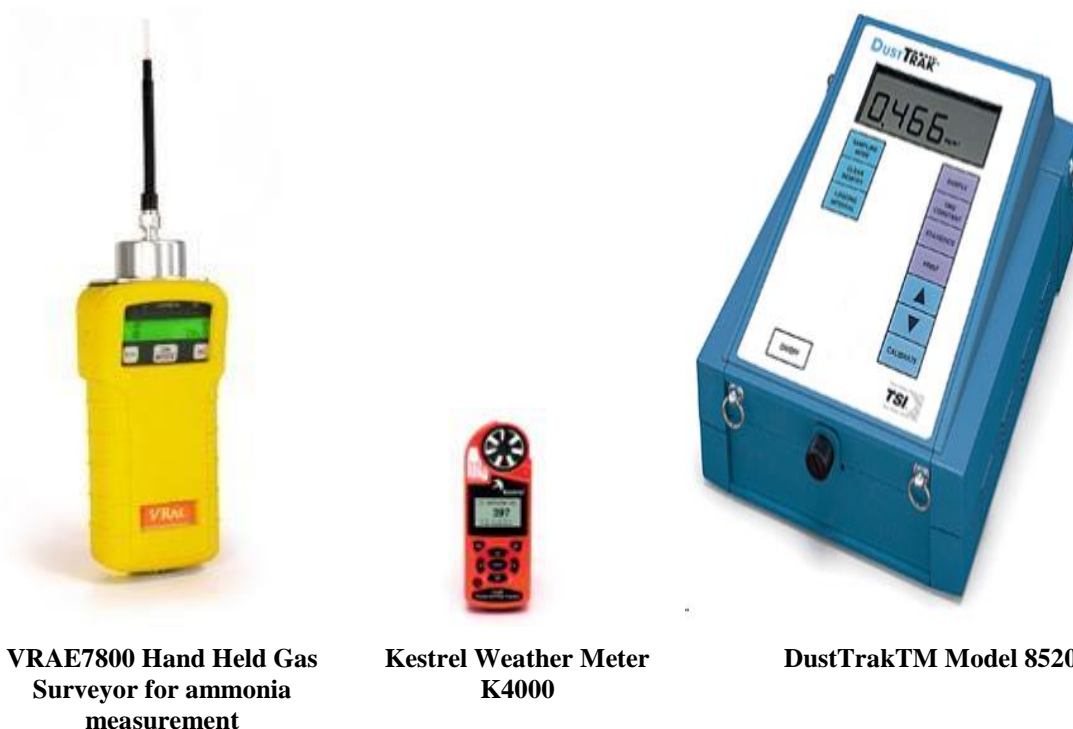


Figure 5-1: Equipment used to measure ammonia, dust and temperature and humidity

The experiment had a 2 × 4 factorial design, with two types of broiler shed (conventional and tunnel ventilated) and four ages of chickens (Weeks 1, 2–3, 5 and 7). All selected sheds were on different farms and all used fresh wood shavings as litter material, following full cleanout.

Within the overall design there were 3 separate studies viz:

Position within shed. Between 11.30 am and 2.00 pm measurements were taken at a fixed height of 30 cm from 10 fixed positions within the shed (Figure 5-2). Ammonia was measured for at least 15 minutes at 3 minute intervals at each location with temperature and wind speed also averaged over this period. The order of measurement of each location was selected at random each time. Spatial measurements were taken in two periods; morning in between 9:00 am and 11:00 am and afternoon between 2:00 pm and 4:00 pm.

Height above the litter. Measurements were taken from nine positions at heights of 5, 30 and 150 cm above floor level (Figure 5-3). Again measurements were for 15 minute periods or more.

Overnight fluctuation. Between approximately 5.00 pm and 7.00 am continuous measurements were recorded at two positions and two heights (30 cm and 150 cm).

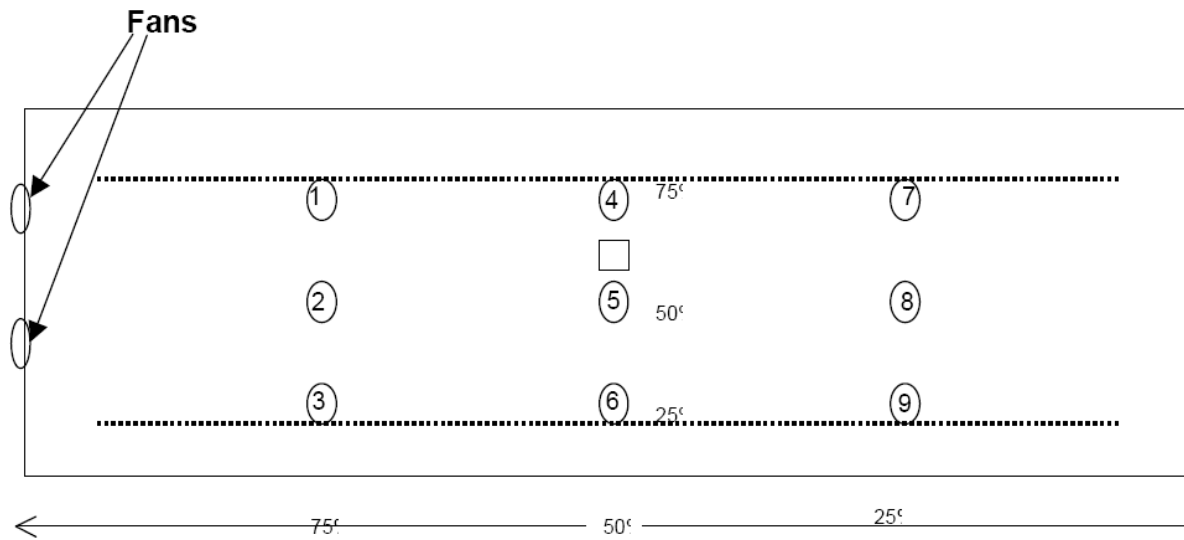


Figure 5-2: Overview of spatial arrangement of sampling sites within the shed. The square (F) represents the proposed sampling site for the future field studies, based on air movement and mixing within a tunnel ventilated shed.



Figure 5-3: Custom made cage for placing three instruments at 30 cm (*left*) or at 5 , 30, 90 and 150 cm above the ground (*right*)

5.2.1 Statistical analysis

For studies 1 and 2, data were averaged for each position and height to provide a single mean estimate for analysis. Position data (Study 1) were analysed by ANOVA for the fixed effects of shed type, position, period (morning and afternoon) and chicken age. Height data (Study 2) were analysed by ANOVA and for the fixed effects of shed type, chicken age, position in the shed and height above the litter. Overnight data (Study 3) were not analysed statistically. Data for each time point were plotted against time to show the NH₃ level in each shed. Similar analyses were conducted for the dust data.

5.3 Results

5.3.1 Ammonia concentration

In study 1 (effect of position) there was a significant effect of age ($P < 0.001$) on the ammonia concentration at 30 cm above the floor without a significant effect of shed type ($P = 0.08$), position ($P = 0.78$) or period ($P = 0.09$). There were no significant interactions between these effects. The mean NH₃ concentrations in both types of sheds at various ages of chickens are shown in Table 5-2. The patterns of ammonia concentration in both shed types at various ages of chickens are plotted in Figure 5-4.

Table 5-2: Study 1. Mean ammonia concentration, temperature, relative humidity and wind speed in tunnel ventilated and conventional broiler sheds containing chickens of various ages.

Tunnel ventilated shed					Conventional shed				
Age (d)	NH ₃ (ppm)	Temp (°C)	RH (%)	Max wind speed. (m/s)	Age (d)	NH ₃ (ppm)	Temp (°C)	RH (%)	Max wind speed. (m/s)
6	0.81	19–32	42–73	0.4	7	2.17	19–32	49–93	0.5
22	12.16	20–26	74–100	0.5	14	6.31	19–28	56–94	0.4
33	12.25	19–27	60–93	0.9	34	12.57	18–25	54–83	0.8
45	9.26	20–25	92–100	1.7	46	9.82	15–24	74–95	1.2

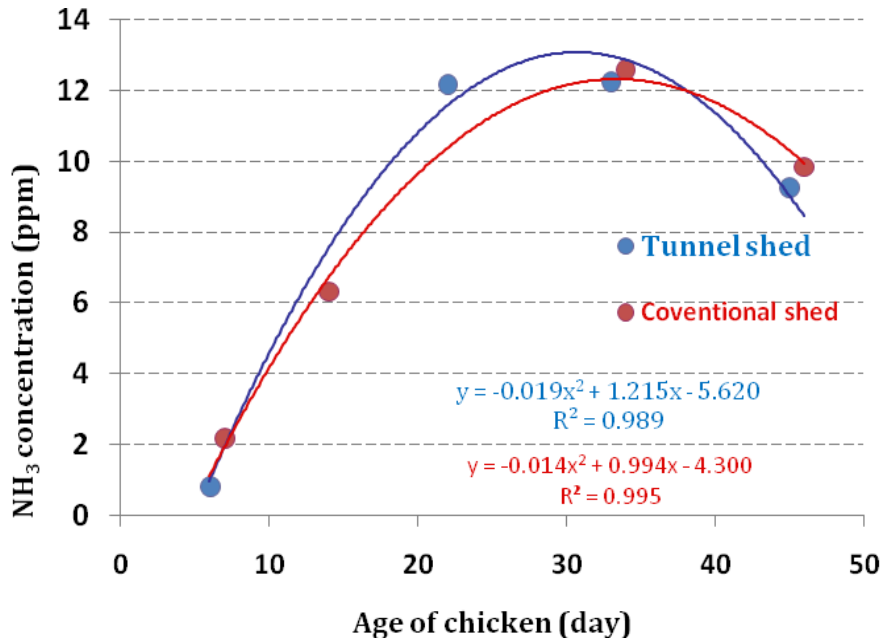


Figure 5-4: Ammonia concentration in conventional and tunnel ventilated shed by the age of chickens. The line fitted is a 2nd degree polynomial

In study 2 (effect of height) there were significant effects of height ($P < 0.001$), shed type ($P < 0.001$) and age ($P < 0.001$) but not position on NH₃ concentrations. There were also significant interactions between the effects of age and height ($P < 0.001$) but not height by shed type ($P < 0.061$) or other interactions. Ammonia concentrations were higher in conventional than tunnel ventilated sheds (9.5 vs 8.4 ppm). No height effect was evident up to week 3, after which NH₃ were significantly higher at 5 cm than any other heights (Figure 5-5).

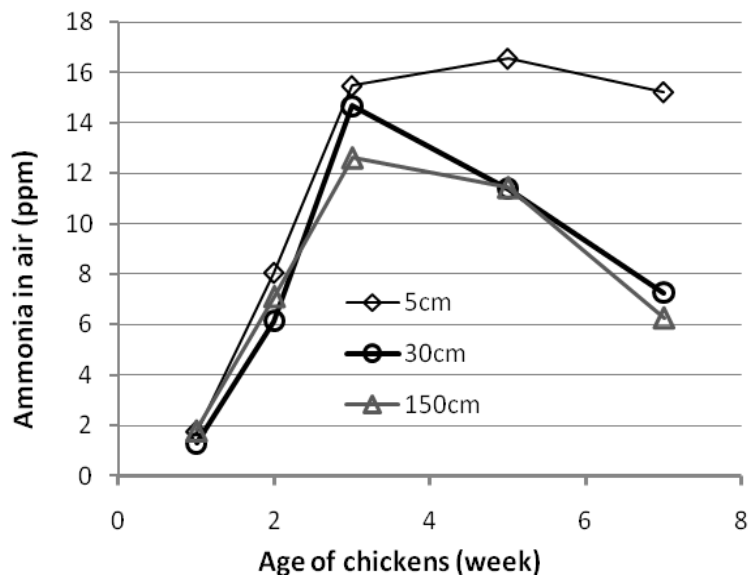


Figure 5-5: Study 2—Interaction between the effects of height of measurement above the floor and chicken age on air ammonia concentrations.

The overnight data (study 3) were plotted against time for shed types (Figure 5-6). There was not much difference in ammonia concentration in conventional and tunnel ventilated sheds, however, the variability of air ammonia in the conventional sheds appeared to be greater than in tunnel ventilated sheds. There was also no discernable systematic variation in ammonia concentrations over the period of measurement, but differences in the age of chickens at sampling were clearly evident.

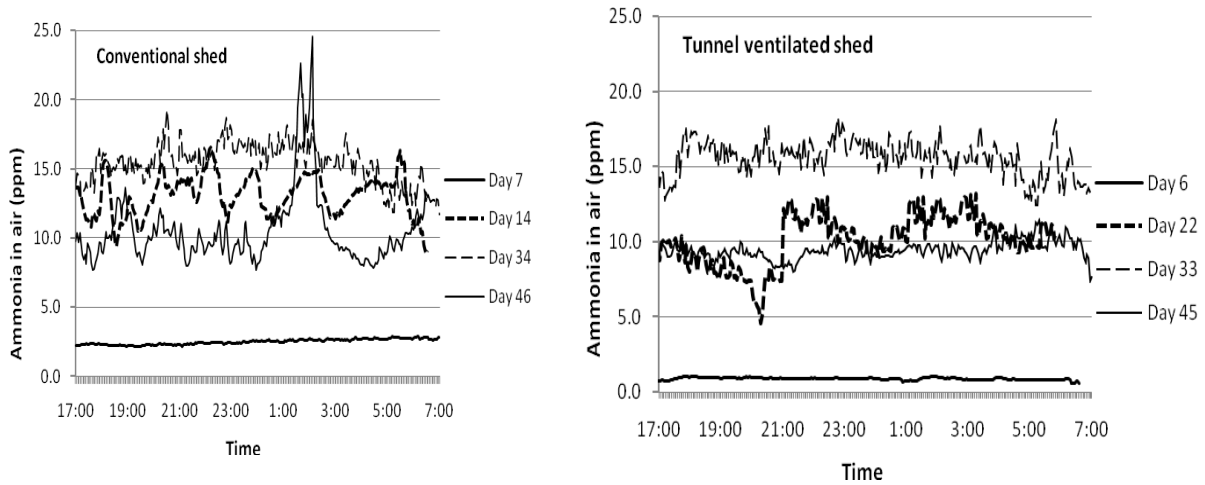


Figure 5-6: Study 3—Overnight air ammonia concentrations in conventional and tunnel ventilated broiler sheds containing chickens of various ages. Data are the mean of two heights (30 and 150 cm).

5.3.2 Dust concentration

For study 1 (effect of position), there was a significant effect of age ($P < 0.001$) but not position ($P = 0.59$) and shed ($P = 0.20$) on the air particulate matter (dust). The dust level was higher in conventional than tunnel ventilated sheds (Figure 5-7). Dust level increased with the age of chickens to around day 35 before declining, presumably due to increased ventilation and removal of chickens at the end of the batch. There was a faster rate of increase in dust concentrations in the conventional sheds.

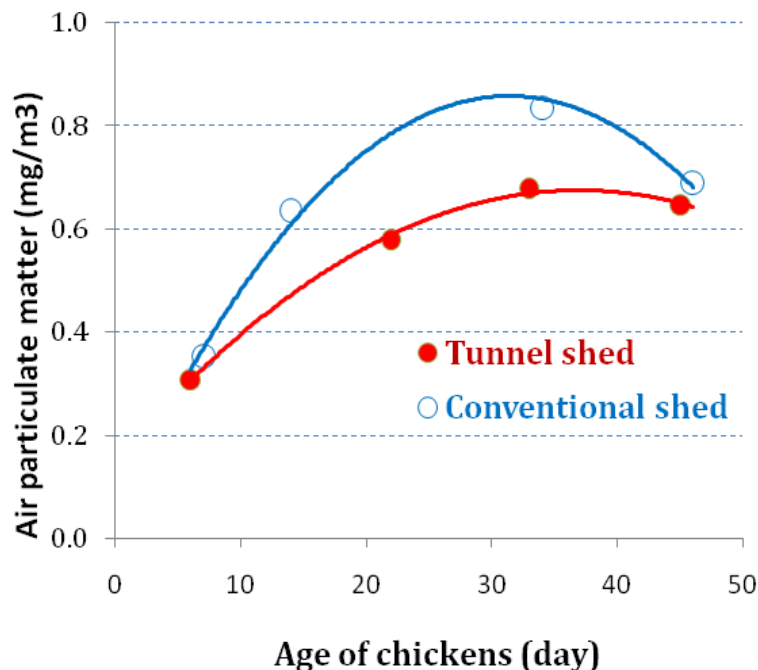


Figure 5-7: Air particulate matter (dust) concentration in conventional and tunnel ventilated sheds at various ages of chickens. The line fitted is a 2nd degree polynomial

In the second study (effect of height), there was significant effect of age ($P < 0.001$), position ($P < 0.001$), and height ($P < 0.001$) with a significant interaction between the effects of position and height ($P < 0.001$). The dust concentration increased with age, and dust concentration was greatest near the floor level (5 cm height) and reduced with height (Figure 5-8). There was some variation in the dust concentration with position, with a lower concentration at position 2 (Figure 5-2) than the other eight positions. The position F was not included in this study.

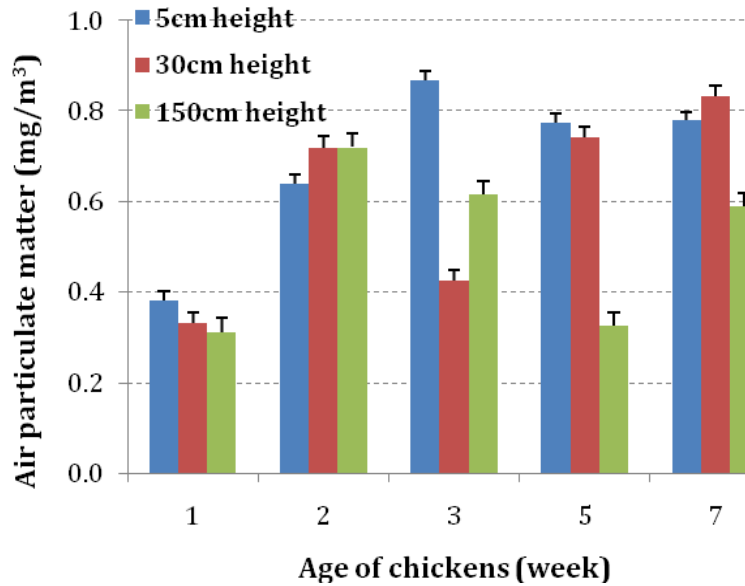


Figure 5-8: Air particulate at various heights at various ages of chickens

5.4 Summary and conclusions

The main purpose of this study was to evaluate spatial variation of NH_3 and dust concentrations in sheds to determine whether single point monitoring in longitudinal studies would be valid.

There was no systematic difference in ammonia concentrations for the chosen 10 positions within the shed indicating that that monitoring of NH_3 concentrations from any of these positions within the shed is representative of the whole shed. However, there was a significant effect of height above the litter, with ammonia concentrations being higher near the surface of the litter (5 cm). The height at which sampling occurs should be selected to represent the air being inhaled by the target population (chickens or people).

The ambient NH_3 concentrations in the eight studied broiler farms were relatively low compared to some overseas studies (eg. Wathes et al., 1997), where in-shed NH_3 concentrations often exceeded 50 ppm. Mean NH_3 concentrations always remained below the threshold value of 25 ppm, with slightly higher NH_3 concentrations in conventional than tunnel ventilated sheds. This may reflect the use of new litter in this study as NH_3 could be expected to be higher on reused litter (Miles et al., 2004).

Air NH_3 concentrations increased with increasing age of the chickens to week 3 then plateaued to week 5 before declining. The plateauing after week 3 is likely due to increased ventilation rates after brooding. The decline after week 5 is likely due to both increased ventilation rates and decreased stocking densities following partial removal of chickens. Overall, we conclude that NH_3 concentrations in Australian broiler production systems based on full cleanout and single use litter are relatively low and the distribution within a shed is homogenous in regard to position, but not height.

With regards dust concentrations, there was significant variation based on location within the shed, and sampling points in the centre of the shed or close to the fans should be avoided. Between 2 and 5

weeks of age dust levels were higher in conventional than tunnel ventilated sheds. As might be predicted, dust levels declined with height above the floor. The measured levels are below the thresholds for time weighted exposure (35 hrs/week) to either softwood (5 mg/m^3) or hardwood (1 mg/m^3) dust in the National Exposure Standards for Atmospheric Contaminants in the Occupational Environment [NOHSC:1003(1995)] although it must be recognised that our dust monitor measures particles up to $10 \mu\text{m}$ only.

6 Comparison of ammonia and dust level in new and reused litter in broiler sheds

Experiment 7. LT09-C-FS2

Start: 07/09

Completion: Ongoing

6.1 Introduction

Ammonia (NH₃) is an unwanted by product of litter decomposition on chicken farms. High NH₃ is detrimental to chicken welfare and productivity, causing reduced feed intake and growth and increased susceptibility to pathogens (Miles et al., 2004; Ritz et al., 2004). The reported concentrations at which adverse affects occur vary but an exposure limit of 25 ppm has generally been set by industry and welfare bodies of different countries on human safety and animal welfare grounds (Kristensen and Wathes, 2000; Wathes et al., 1983). Ammonia concentrations in broiler sheds depend on many factors including litter moisture level, pH, ventilation rate and chicken age.

As ammonia is produced by bacterial de-amination of nitrogenous substances it could be expected that the increased load of faecal matter in reused litter, particularly during early batch, would increase ammonia production compared to new litter, and indeed this appears to be the case (Miles et al., 2004). This is widely accepted in industry and in countries such as the USA where litter reuse is common and consequently there is a significant industry producing litter amendments to reduce ammonia production from used litter. Anecdotally in Australia there can be ammonia problems on reused litter early in the batch when conditions are warm and ventilation in the shed is reduced.

In order to evaluate ammonia production on new and used litter under different seasonal and other conditions, we designed a series of longitudinal studies with two poultry companies. Equipment was purchased and a design established based on the results of Chapter 5. However, to date we have had limited data returned from the field due to equipment calibration problems, staffing issues and in some cases problems with downloading information from the monitoring equipment.

This short chapter reports on the data we have at hand at the time of reporting.

6.2 Materials and Methods

The experiment was designed to monitor ammonia concentration in two companies, located in Sydney (Cordina Poultry Farms) and Brisbane (Inghams Enterprises) regions; one using new litter and another one reused litter with same type of shed (conventional in Sydney and tunnel shed in Brisbane) covering a complete year (all four seasons). However, due to the technical problems noted above, complete sets of measurements were received from only two farms in Sydney area for one batch of chickens, one with new and another with reused litter (complete reuse including brooder area). Both farms had conventional sheds and data were from July–August 2009.

Aerial ammonia concentrations were measured using VRAE7800 Hand Held Gas Surveyors (Geotech Environmental Equipment, Inc., Colorado, USA) calibrated against a 50 ppm NH₃ standard sample (Figure 5-1). Wind speed, humidity and temperature were recorded with Kestrel Weather Meter K4000 (Nielsen-Kellerman, Inc., PA, USA).

Particulate matter in the shed air was measured (mg/m³ air) using DustTrak™ Model 8520 aerosol monitors (TSI Inc, Minnesota, USA). The DustTrak measures dust particles ranging from 0.1–10 µm using light scattering technology with a laser photometer. A complete description of the machines was provided in Chapter 5. The machines were placed in the position F as shown in Figure 5-2 at a fixed

height of 30 cm from above the floor. Data were recorded every three minutes for 16–22 hours at various stages during the batch.

6.2.1 Statistical Analysis.

The age (in days) was classed into week categories for analysis. Analysis of variance was performed for the effect of age (week) and litter type (confounded with farm), and their interaction.

6.3 Results

6.3.1 Ammonia

There was significant effect of age ($P < 0.001$) and litter ($P < 0.001$) with a significant interaction between the main effects ($P < 0.001$) on the shed ammonia concentration. Overall ammonia concentrations were higher in the reused litter (7.0 ppm) sheds than new litter shed (3.4 ppm). Ammonia concentration decreased with age of chickens in both litter type from week 3 (Figure 6-1 and Figure 6-2).

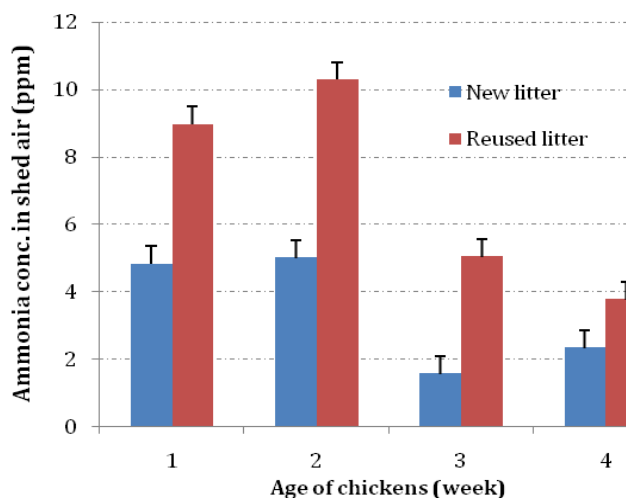


Figure 6-1: Interaction plot showing the effects of chicken age and litter reuse on air particulates

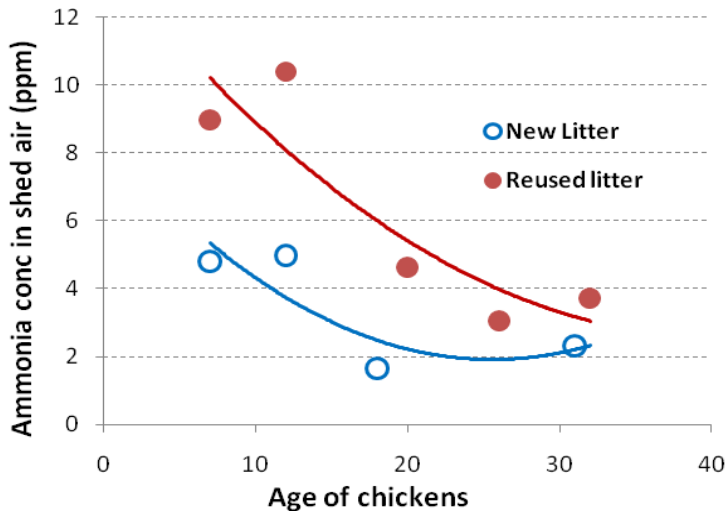


Figure 6-2: The same data as for Figure 6-1 shown on a scatter plot with actual age of chicken in days. The curve is a 2nd degree polynomial fit.

6.3.2 Dust

There was a significant effect of litter type ($P < 0.001$) and chickens age ($P < 0.001$) with a significant interaction between the main effects ($P < 0.001$) on the particulate matter in the broiler shed. The particulate matter content of air increased with the age of chickens and overall a higher particulate concentration was measured with reused litter when compared to new litter (Figure 6-3 and Figure 6-4). The increase in the air particulate matter was more rapid with reused litter compared to new litter, however, by week 5 dust levels were higher on new than reused litter.

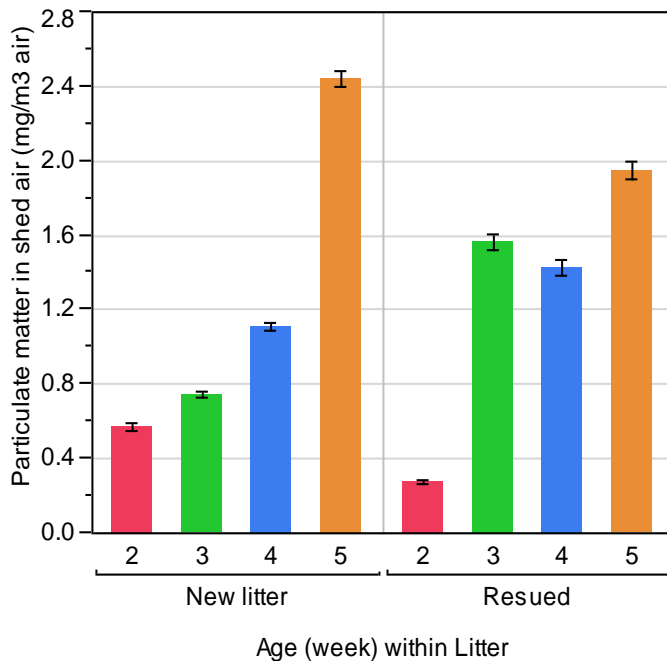


Figure 6-3: Air particulate matter (dust) level of broiler chickens on new or reused litter at various ages.

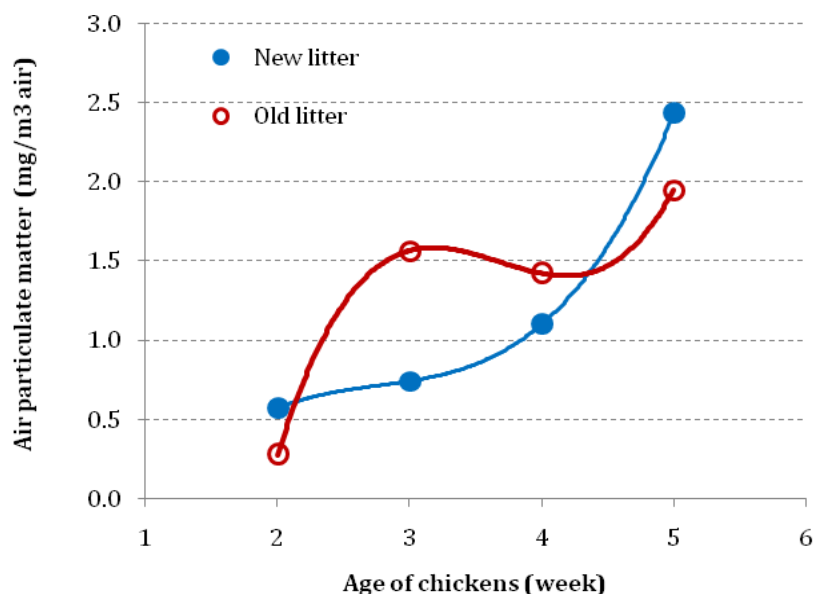


Figure 6-4: Change in air particulate matter (dust) level over time in sheds containing broiler chickens on new or reused litter. The curve is a 3rd degree polynomial fit.

6.4 Summary and conclusions

This main finding at this stage of the longitudinal study is that although there was a significantly higher ammonia concentration in the reused than the new litter, the concentration of ammonia was well below the threshold value of 25 ppm throughout both batches.

The dust level was also somewhat higher in the reused litter, particularly in weeks 3 and 4, after which dust levels from new litter exceeded those from old litter. The dust level measured in these conventional sheds are consistent with the respirable dust content reported for European broiler (0.42–1.14 mg/m³) flocks and the inhalable dust content of 3.8–10.4 mg/m³ in the same study (Wathes et al., 1998). They are below the thresholds for time-weighted exposure (35 hrs/week) to softwood (5 mg/m³) but not hardwood (1 mg/m³) dust in the National Exposure Standards for Atmospheric Contaminants in the Occupational Environment (NOHSC, 1995). However, it should be noted that measurements included dust particles only up to 10 µm in diameter. Furthermore measurements were made at 30 cm height where dust concentrations could be expected to be much greater than at human head height.

The final dataset in this experiment is disappointing in size given the objectives of the study and the resources provided in terms of monitoring equipment. It emphasises the difficulties of conducting on farm research with the companies supplying the labour required.

7 Discussion of Results

7.1 Progress against agreed milestones.

The project, which ran for 2.5 years, successfully achieved the milestones set, as summarised below.

Milestone 1. Literature review and risk assessment (31/3/08)

This milestone was delayed but met, and an improved version of the literature review and risk assessment is appended to this report.

Milestone 2. Review project case for continuation (30/6/08)

This milestone was met on time with the Industry Steering Committee recommending continuation of the project, subject to some changes in the nature of the experiments to be carried out (more emphasis on ammonia and field studies).

Milestone 3. Develop protocol for chick bioassay for key viral pathogens (30/11/08)

This milestone was met on time and a useful chick bioassay developed (Chapter 2). This has been published in conference form (Islam et al., 2009) and was used successfully in subsequent experiments testing the effects of litter treatments on viral pathogen carryover (Chapters 3 and 4).

Milestone 4. Develop litter sub-sampling and transportation protocols (30/11/08)

This was achieved on time, with the protocols developed as part of the work in Chapter 2, and validated in Chapters 3 and 4.

Milestone 5. Completion of two experiments on pathogen survival (30/4/09)

This was achieved slightly late, with two major experiments on pathogen survival in heaped or windrowed litter, one in Sydney (Chapter 3) and one in Brisbane (Chapter 4). Associated with each of these experiments was a separate experiment to produce positive control litters and test the effects of transportation, relating to Milestone 4.

Milestone 6. Completion of two further field experiments (30/10/09)

This was achieved with a major study on spatial and temporal variation in ammonia and dust levels during chicken batches in conventional and tunnel ventilated sheds (Chapter 5) in April–June 2009, and a less successful ambitious longitudinal field study to compare ammonia and dust levels on farms using new and reused litter throughout the year (Chapter 6).

7.2 Summary of project activities

The overall objective of the project was to:

Determine survival times of key viral poultry pathogens in litter under a variety of litter management practices; and

Develop specific methods of litter treatment and management to enable safe reuse of litter by broilers under typical Australian conditions.

The project proposed to fulfil these objectives in a phased way by doing the following:

Comprehensively reviewing existing information on the subject including a risk assessment on the pathogens and practices most likely to threaten chicken health, welfare and performance;

Developing and validating novel methods for measuring viral pathogen load in litter; and

Conduct research to devise optimum methods for litter reuse taking into account the geographical, breed, nutrition and litter type variation that exists within the Australian chicken meat industry. This work will specifically involve:

testing the effects of a range of litter partial composting treatments on the temperature, pH and chemical composition of litter on commercial farms;

testing the effect of these same treatments on survival/infectivity of viral pathogens and coccidia in the litter; and

investigation into the temporal and spatial distribution of ammonia in sheds, and the effects of litter reuse on ammonia production.

Progress against each of these latter more specific objectives is discussed below.

7.3 Achievement of project objectives

Objective 1. Comprehensively review existing information on the subject including a risk assessment on the pathogens and practices most likely to threaten chicken health, welfare and performance

A 59-page literature review and risk assessment is appended to this report. It includes a 2007 survey of poultry companies on their litter use strategies. The survey revealed that the main litter type used in Australia is wood shavings (up to 50%), saw dust (up to 30%), rice hulls (15%), straw (<05%) and shredded paper (<01%). The cost of litter varies from \$12 to \$21 per cubic metre, depending on type and availability. In general 'wood shavings' is the most expensive litter followed by 'sawdust' and 'rice hulls'. The litter materials are most expensive in Queensland and cheapest in Tasmania and Victoria. Two surveyed companies use reuse litter for multiple batches on a portion of their broiler farms. The major reuse practices are full reuse with partial composting via in-shed heaping, partial cleanout with reuse of the grower area and full cleanout of the brooder area between batches. Fertilizer is the only use of spent litter. Some growth in partial cleanout over the in last five years was reported in the survey.

The literature review revealed that the majority of the poultry pathogens, for which there are concerns when reusing litter, are viral. While there is considerable information available on bacterial survival in poultry litter and its consequences on the subsequent batches, literature on the survival of viral pathogens in poultry litter is limited. Despite the consensus that litter reuse increases disease risk, there are reports from Australia and overseas that chickens on reused litter perform better than on new litter. That suggests that reusing litter in the poultry shed may not necessarily compromise bird health and welfare, if sound litter management practices are adhered to. On the other hand there are also reports that reusing litter increases ammonia, odour and dust emission and can have adverse effects on chicken health and productivity. Maintaining litter dryness and friability is the single most important factor for both animal health and food safety, and litter reuse should only be considered if litter conditions are appropriate.

There are three major practices used to manage multiple batch litter between chicken batches. These are no treatment (except removal of cake), partial composting via heaping of litter within the shed, or the addition, generally sprinkled on the surface of the litter, of chemical and/or microbiological additives to reduce ammonia production and possibly pathogen survival. Litter may be reused throughout the shed, or only in the grow out area, with the brooder area subject to full cleanout and new litter. Use of litter additives has not been practiced in Australia to date, perhaps for economic reasons, but it is widespread in other countries where litter reuse is practiced.

Based on the available literature and industry practices, the risks associated with litter reuse for each of the major viral diseases of chickens in Australia are categorized as 'high risk', 'low risk' and 'very low risk'. High risk diseases, for which litter reuse may exacerbate the disease risk are infectious bursal disease, Marek's disease, chicken infectious anaemia and fowl adenovirus (including body hepatitis/ hydropericardium syndrome). Other avian health problems such as breast blister, hock burn, pododermatitis, necrotic enteritis, cellulitis are also categorised as 'high risk' problems related to multiple batch litter reuse, although in reality their incidence is related to overall litter management regardless of reuse status. Newcastle disease virus, infectious bronchitis virus, infectious

laryngotracheitis virus are classified as 'low risk' pathogens. Fowl pox virus, avian reovirus, avian influenza virus (low pathogenic) are classified as 'very low risk' pathogens related to litter reuse.

In practice, the poultry health risks associated with litter reuse could be greatly reduced by careful assessment of the risk of reuse for each litter with preparedness to move to full cleanout when litter conditions or flock health status and performance indicate it. The risks may also be ameliorated by sound litter treatments between batches. In Australia, partial composting via heaping of litter appears to be the most viable option in this regard, having a proven track record. However, other treatments should not be dismissed as economies of scale may make them economically feasible if widely accepted. An important component of testing the efficacy of such treatments is being able to assess their effect on survival of viral pathogens in litter. The development of new tests to enable this to be done could facilitate such testing and enable decisions on litter reuse for chickens to be placed on a more rational footing.

Objective 2. Develop and validate novel methods for measuring viral pathogen load in litter

Viruses can only be grown in living media, so routine isolation from filthy litter material is not a practicable means of monitoring viral load in litter. Direct molecular detection of virus is possible, but may not be an accurate measure of infective virus, as viral DNA/RNA may persist in the absence of infectivity. To determine whether viral load is reduced by the end of batch litter treatments employed for this purpose a means is required of measuring reductions in infective load in litter.

Our approach to resolving these issues was to develop a chick bioassay and this is detailed in experimental Chapter 2. This was successfully achieved. Key issues and discussion points arising from this work are discussed below.

The type and age of chicken to use. Our experiments clearly showed that day old SPF chickens are the ideal chicken for the bioassay. Fears of widespread disease morbidity and chick mortality have not been realised, with very low mortality rates observed, generally 1–2% over the 35–42 day test period. We clearly showed that commercial broiler chicks are unsuitable due to significantly greater mortality problems (do these birds carry the disease organisms with them from the hatchery?) and the presence of maternal antibody that inhibits or obscures the serological response to some pathogens. With regards chick age, it was shown that SPF chicks aged 0 and 8 days at initial exposure to reused litter showed no difference in response.

End point and qualitative/quantitative nature of the bioassay. The end point selected initially, seroconversion at day 35 post exposure to litter (based on the chick inoculation test), has proved successful. Furthermore it is clear that quantitative outcomes are achieved, with significant differences in the proportion of chicks seroconverting correlating well with proposed changes in litter infectivity due to heaping or other treatments. In some cases, analysis of mean titre, in addition to the proportion of positive chicks provides a quantitative outcome.

Ability to transport litter to the bioassay site. In 3 major experiments we have confirmed that transport simulation for 24 hours does adversely affect litter infectivity. The data vary somewhat on this point, and there may be some minor loss of infectivity for susceptible viruses (eg Infectious bronchitis virus), but for the majority there appears to be no effect of transportation using the protocols provided.

Issues remaining with the bioassay. The main outstanding issues with the bioassay include:

- Cost. This is an expensive test.
- Absence of pathogens in field litter. A great deal of expense can go towards litter treatments and sampling in the field, only to find many pathogens missing in the samples, so that the effect of treatment on pathogen survival cannot be ascertained.
- Inconsistent results. While results for some fairly ubiquitous viruses (eg. MDV, FAV, CAV) generally show clear effects of treatment, for some viruses occurring at lower levels, positive

infectivity can be detected following negative samples, or infectivity can increase unexpectedly, rather than decrease. At least part of this problem is almost certainly due to chance while sampling. In Chapters 3 and 4 it is clearly demonstrated that the surface of heaps and windrows remains comparatively cool, and therefore a reservoir of infective material is likely to remain. It is during sampling from near the surface of the pile that “hot spots” of infectivity may be sampled, even as late as 10 days after heaping/windrowing.

Ideally the chick bioassay can be used to validate a cheaper and easier test of viral infectivity such as direct molecular detection in litter.

Objective 3. Test the effects of a range of litter partial composting treatments on the temperature, pH and chemical composition of litter on commercial farms

This objective was realised in Chapters 3 and 4 in which completely novel information was obtained on the dynamic change in litter over time when it is heaped and windrowed. Due to the role of temperature in pathogen inactivation, this is probably the variable of greatest interest. The main findings from our experiments in this regard were:

a) Depth in the litter stack has a profound effect on the temperature profile.

- The surface remains at little above ambient temperature and may harbour a potential reservoir of infective pathogen in unturned stacks.
- At 25 cm depth, there is a very rapid rise 60°C or higher (days 2–3) but temperatures decline slowly thereafter to 55–60°C at days 9–10.
- At 75–100 cm depth, the rise in temperature is very much slower but temperatures continue to increase through to day 10 to 55–65°C. Final temperature is higher in heaps than windrows. During the first few days of composting, the material at 25 cm can be 10–15°C hotter than this deep material.
- At 50 cm depth an intermediate pattern is apparent with temperatures peaking at around day 7 and plateauing after that. Temperatures from day 5–6 onwards were highest in this stratum and are almost always above 60°C.

b) Heaps achieve higher temperatures than windrows (Chapter 3).

Up to day 5 there is little difference between heaps and windrows, but beyond this windrows begin to cool while heaps maintain temperature overall.

c) Turning increases post-turn temperatures in heaps but not windrows (Chapters 3 and 4).

d) Depth and turning have minor effects on litter left on the ground (Chapter 4).

There is a temperature gradient in un-heaped litter with a range of 5–10°C between the cool surface and the temperature at the base of the litter. Turning of the litter on the floor has no appreciable effect on these temperature profiles.

With regards dry matter, pH and litter chemistry (Chapter 3) there are few major findings. The main findings from our experiments in this regard were:

- Litter has an alkaline pH (pH ~8.6).
- Litter pH declines with time in heaped/windrowed litter (by 0.2–0.4 units).
- Litter pH is highest at the surface and fairly uniformly lower at 25–100 cm in the stack.
- Dry matter increases with time post heaping/windrowing (from ~74% to ~77% by day 9).
- Dry matter decreases with increasing depth from the surface (from 79% to 73%).

- There was a significant positive association between the dry matter content and litter pH ($R^2=0.33$, $P<0.001$) and negative associations between dry matter and nitrogen content ($R^2=17.9$, $P<0.001$), pH and nitrogen content ($R^2=0.18$, $P<0.001$).

Objective 4. Test the effect of the litter treatments on survival/infectivity of viral pathogens and coccidia in the litter

This objective was met in the experiments described in Chapters 3 and 4. The general hypotheses under test in these experiments were:

Infectivity of litter for viruses and coccidia will be reduced following heaping or windrowing;

Infectivity will be reduced more in treatments generating greater temperatures;

Heaping will be more effective than windrowing;

Turning will lead to increased inactivation; and

Most of the benefits will be seen by day 3.

The findings will be reviewed in light of each of these.

1. Infectivity of litter for viruses and coccidia will be reduced following heaping or windrowing.

Our findings were that coccidial oocysts were readily inactivated by day 3 in experiment 1. Coccidia are known to be sensitive to high temperatures and ammonia concentrations and these are no doubt responsible for this effect. FAV was largely inactivated by days 6–7 (with some +ive at day 10) while CAV and IBDV were largely inactivated by days 6–10 (with some +ive at day 10). MDV showed uniform reductions in infectivity over time but significant infectivity remained at days 9–10. Unlike the other viruses, MDV inactivation was not affected by treatment, so appeared to be a time effect, rather than a treatment effect.

2. Infectivity will be reduced more in treatments generating greater temperatures. This was generally true for CAV for which significantly greater inactivation was seen in heaps than in windrows. There was a non-significant trend in this direction amongst some of the other viruses, but not MDV.

3. Heaping will be more effective than windrowing. This hypothesis is related to hypothesis 2 and is supported for CAV, with some support from other viruses such as FAV.

4. Turning will lead to increased inactivation. There was no evidence to support this in the data. Indeed for CAV turning tended to the degree of inactivation obtained in both Chapter 3 and 4.

5. Most of the benefits will be seen by day 3. There was little evidence to support this, apart from the case of coccidia and rare occasions when a very high level of viral inactivation was achieved by day 4 (eg CAV in Chapter 4 and FAV in Chapter 3). In general, benefits in terms of inactivation continued to increase with increasing time, up to days 9 and 10.

Objective 5. Investigate the temporal and spatial distribution of ammonia in sheds, and the effects of litter reuse on ammonia production

This objective was dealt with in Chapters 5 and 6 with the inclusion of dust also in these experiments. There were some ammonia measurements also made during the litter treatment period in Chapter 3, at a time when levels may cause OH&S concerns for staff rather than chickens.

With regards spatial variation in ammonia in broiler sheds, there was no systematic difference in ammonia concentrations for the chosen 10 positions within the shed indicating that that monitoring of NH_3 concentrations from any of these positions within the shed is representative of the whole shed.

However, there was a significant effect of height above the litter, with ammonia concentrations being higher near the surface of the litter (5 cm).

The ambient NH_3 concentrations in the eight studied broiler farms were relatively low compared to some overseas studies and mean HN_3 concentrations always remained below the threshold value of 25 ppm, with slightly higher NH_3 concentrations in conventional than tunnel ventilated sheds.

Air NH_3 concentrations increased with increasing age of the chickens to week 3 then plateaued to week 5 before declining. The plateauing after week 3 is likely due to increased ventilation rates after brooding. The decline after week 5 is likely due to both increased ventilation rates and decreased stocking densities following partial removal of chickens.

Overall, we conclude that NH_3 concentrations in Australian broiler production systems based on full cleanout and single use litter are relatively low and the distribution within a shed is homogenous in regard to position, but not height.

With regards dust concentrations, there was significant variation based on location within the shed, and sampling points in the centre of the shed close to the fans should be avoided. Between 2 and 5 weeks of age, dust levels were higher in conventional than tunnel ventilated sheds. As might be predicted, dust levels declined with height above the floor. The measured levels are below the thresholds for time weighted exposure (35 hrs/week) to either softwood (5 ppm) or hardwood (1ppm) dust in the National Exposure Standards for Atmospheric Contaminants in the Occupational Environment [NOHSC:1003(1995)].

In Chapter 6, initial data comparing ammonia concentration in shed with new and reused litter showed that ammonia concentrations were indeed higher during the first 3 weeks on used litter, but that concentrations remained relatively low, well below the 25 ppm threshold.

In Chapter 3 ammonia was measured in naturally ventilated broiler sheds during de-caking operations as well as during and after heap formation. Continuous measurement by the ammonia meter enabled assessment of peak and short-term steady values for airborne ammonia concentration. At all times, the sidewall curtains on the sheds were open and a slight breeze was blowing (measured to be 0–2.0 m/s on the inside of the shed).

On the upwind side of the shed or litter heaps, ammonia concentration rarely exceeded 2 ppm even when the exhaust air from upwind sheds were blowing in. On the downwind side of the heaps, ammonia concentration reached approximately 26 ppm when positioned within 0.3 m of the heap. Once the meter was repositioned to be 1.0 m from the heap, ammonia concentration reduced to approximately 10 ppm. During de-caking and heap forming operations, disturbance of the litter caused the ammonia concentration to increase to approximately 35–38 ppm; however, these peaks only occurred for very short periods.

The Australian Government has recommended safe workplace exposure limits for ammonia (Australian Safety and Compensation Council 1995). These are given in terms of an eight hour time weighted average (TWA) exposure of 17 mg/m^3 (approximately 25 ppm) and a fifteen minute short term exposure limit (STEL) of 24 mg/m^3 (approximately 35 ppm). When compared to safe workplace levels, the concentration of ammonia measured at this farm occasionally exceeded the levels listed for the time weighted and short term exposure limits (TWA and STEL). On this occasion, no attempt was made to measure the actual exposure for the grower (or machinery operator) for the durations specified by TWA and STEL. However, because the concentration of ammonia exceeded those corresponding to TWA and STEL, there is a risk of excessive exposure and growers should assess the risk of excessive ammonia exposure for themselves and workers, especially while de-caking litter or forming, turning or spreading the heaps.

8 Implications and impact of the findings for the Australian Industry

The main implications for industry are as follows:

Confirmation of carryover of viral pathogens in litter. Placing birds on untreated litter remains a risk.

Confirmation that partial composting treatments will greatly reduce the infective load of most pathogens, and may even eliminate it in some cases.

Clear findings regarding litter treatments:

- The bigger the heap the better (although slower to heat).
- The surface remains a significant reservoir of unheated material.
- Turning appears not to confer a major benefit despite the point above.
- In most cases, by day 3–4 significant reduction will have occurred in pathogen load but significant further reductions can be expected with increased time.

Findings regarding ammonia:

- Litter reuse will increase ammonia, but in none of our studies has ammonia approached levels likely to adversely affect chickens.
- Methodology for in shed monitoring experimentally established.
- Potential for adverse exposure to humans working with heaps and windrows.

Review of the literature and risk assessment:

- Clear banding of viral pathogens into 3 risk categories for litter transmission
 - High risk - infectious bursal disease, Marek's disease, chicken infectious anaemia and fowl adenovirus (inclusion body hepatitis/ hydropericardium syndrome).
 - Low risk - Newcastle disease virus, infectious bronchitis virus, infectious laryngotracheitis virus.
 - Very low risk - Fowl pox virus, avian reovirus, avian influenza virus (low pathogenic)

This banding is largely supported by our experimental findings

Increased capacity to manage litter treatment:

- Greatly improved understanding of temperature and chemistry dynamics in heaped litter.
- New tool for measuring change in litter infectivity.
- Sufficient information available for basic risk assessment on an individual shed basis.

Essentially this project has positioned the industry to make clearer more rational management decisions regarding litter reuse as economic pressure to do so mounts. This is because of the detailed experimental data produced.

9 Recommendations

The major recommendations arising out of the activities of the project to further develop, disseminate or to exploit commercially the results of the Project are as follows:

Facilitate wide dissemination of project findings, particularly with regard to temperatures in litter heaps and windrows and viral inactivation rates.

Support ongoing work in this area to:

- Develop a detailed understanding of the interactions between temperature, ammonia level and duration of exposure on inactivation of the main pathogenic poultry viruses of concern in Australia. This will require use of artificial challenge and *in vitro* simulation of litter heap conditions. We are now equipped to do this with the data arising from this project.
- Develop and validate direct methods for determining viral pathogen load in litter that do not require the bioassay. With the development of an effective (but expensive) bioassay, it is now possible to validate such methods, when it would have been difficult in the past. We consider it likely that molecular methods will prove effective in this regard.
- Investigate methods for ameliorating or managing ammonia production from reused litter under Australian conditions. Successful uptake of litter reuse will probably require that both the pathogen issue and the ammonia issue are resolved such that they can be effectively managed.

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