Final report summary

Population-level biomarkers of gut health in commercial flocks
Abstract
Maintaining chicken gut health is considered to be of major importance for bird welfare and productivity. However, there is a lack of practical and easy-to-apply methods to objectively assess gut health on-farm. The main purpose of this project was to evaluate the application of non-invasive population-level biomarkers to monitor gut disruptors and microbiota composition in commercial chicken flocks. Non-invasive and easy-to-collect population-level biomarkers, such as poultry dust and pooled excreta, do not require terminal sampling and have the potential to provide flock-level metrics on microbial composition and burden of enteric pathogens using fewer samples and therefore at less cost. These tools, when established, could provide timely information that would help with the identification, implementation and evaluation of targeted interventions.

Background
The increasing restrictions on the use of antimicrobials in meat chicken production have resulted in the re-emergence of enteric diseases such as coccidiosis and necrotic enteritis, and the emergence of other less-defined intestinal disturbances that have been linked to shifts in the gut microbiota. Current practices for monitoring these conditions are usually based on post-mortem evaluation and scoring of gut lesions, but this method is unable to detect subclinical infections, has no predictive value and requires skilled personnel as the scoring can be subjective.

The monitoring of markers for enteric pathogens and gut microbiota composition could potentially facilitate the detection of enteric disturbances prior to the occurrence of clinical signs, and inform management interventions on-farm – but currently there are no practical methods for monitoring these conditions in commercial farms.

The assessment of the gut microbiota in particular is usually restricted to research settings because of the high cost of analysis and bird-to-bird variability within flocks. The use of non-invasive samples that are representative of the chicken flock, such as pooled excreta and dust, offers advantages compared with traditional monitoring methods based on individual birds. These advantages include ease of collection and the need for fewer samples, and the lower cost associated with collecting non-invasive samples makes them more suitable as a tool for use in commercial farms. Such tools have the potential to provide flock-level metrics on microbiota composition and burden of enteric pathogens.

Objectives
The broad objective of this project was to evaluate and identify practical and affordable tools to detect gut pathogens and microbiota in non-invasive flock-level samples. The specific objectives were:

1. Evaluate the usefulness of a molecular-based test to detect different coccidia species and the causative agent of necrotic enteritis, Clostridium perfringens, and associated virulence factor netB plasmid in dust and pooled excreta, and their association with flock productive performance.

2. Establish the microbiota profiles in pooled excreta and dust, and their association with flock productive performance.

Research
This study was designed to investigate a) the association of the genomic material of important gut disruptors such as coccidia and the causative agent of necrotic enteritis in dust and pooled excreta samples and flock performance; and b) the bacterial signatures of high and low-performing commercial meat chicken farms in dust and pooled excreta samples. The project was carried out over two phases.

In Phase 1, settled poultry house dust and pooled excreta from the floor were collected weekly at days 7, 14, 21, 28 and 35 of chicken age from 16 flocks at eight farms managed by two Australian integrator companies. In Phase 2, dust samples were collected at 14 and 35 days of chicken age from 68 flocks at 29 farms managed by three Australian integrator companies. The farms were ranked as high or low-performing based on adjusted feed conversion rates. The samples from Phase 1 were tested for the presence of genomic material of Clostridium perfringens and associated netB plasmid, and five coccidian species (Eimeria brunetti, E. maxima, E. necatrix, E. acervulina and E. tenella). The microbial composition of samples from phases 1 and 2 were assessed using 16S ribosomal RNA gene amplicon sequencing. The results were then correlated with each flock’s production data.

Outcomes/key findings
The main findings of this study were:

• Molecular-based testing of dust samples or pooled excreta provides reasonable flock-level measures of coccidia species. If samples are collected at 14-21 and 28-35 days of the production cycle, they allow inference on the peak of coccidia detection and whether an intervention is needed. Pooled excreta seem to offer a more accurate assessment of coccidia load, especially late in the cycle, compared with dust samples, but the latter offers a sensitive screening assay and was considered easier to collect by the farm staff. Dust samples are best collected in settle plates or funnels as scrapped dust samples may contain oocysts derived from previous flocks. Coccidia load in dust is not influenced by location of sample collection within the shed.

• Molecular-based testing of dust samples could be used to screen for the bacterium that causes necrotic enteritis and associated netB toxin plasmid, although the detection of these was not associated with production outcomes in this study.

• The assessment of microbiota in flock-level samples revealed that differential microbiota could be detected in high and low-performing farms within the same company, but that those markers were not stable across companies or within different batches of birds within the same company. This study provided baseline information regarding the microbiota associated with flock productive performance in dust and pooled excreta, which warrants further validation.

As a result of these findings, continued support for the development of practical tools to monitor gut health that could be suitable for use in commercial farms is recommended.
Implications for industry

Monitoring of chicken gut health is considered of utmost importance to ensure bird welfare and to prevent losses in production. However, no practical and objective markers of gut health have been definitively associated with production outcomes to date. This project described easy and practical sampling and analysis methods using dust or pooled excreta to monitor for coccidia and the bacteria that causes necrotic enteritis in meat chickens. These methods have been shown to be potentially useful for monitoring or screening of these pathogens in commercial flocks. The relationship between coccidia load and production outcomes would need to be further assessed in flocks with variable disease status.

This project also explored the use of flock-level sampling to evaluate microbiota associated with production outcomes in different companies. Although there was no consistent microbiota associated with high and low production across companies and flocks, this methodology could be further explored when aiming to detect specific microbial taxa, e.g. when evaluating the administration of specific probiotic strains in commercial flocks.

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Publications

