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Research Visit Grant

1. Key details

[redacted]
Project title: Exploring the impact of the phytochemicals; quercetin and berberine, on the chicken bacteriome and resistome; using a cecum fermenter model.
Project start date: 06/07/2020
Project end date: 31/07/2020
Expected costs: £1920.00
Maximum we can award: £3000.00
Breakdown of costs:

Flights from Liverpool to Amsterdam:	£150
Accommodation in Almere (AirBnB) for 4 weeks:	£1500
1-month rail pass Almere to Lelystad:	£7 / day = £280 / Month
Total:	£1930

2. Project details

Project host: [redacted]
Host Institute: Wageningen University & Research | WUR · Wageningen Bioveterinary Research
Project location: [redacted] Netherlands

2.1 Project description

This project will determine the impact of two phytochemicals, quercetin and berberine, on the caecum microbiome, and its cognate resistome, using a chicken caecum fermenter model.

Two experiments will be conducted over four weeks. This experiment aims to capture the effect of plant metabolites on a developing, and developed caecal microbiome, respectively.

Samples extracted from these experiments will be analysed using direct culture to determine if there is a change in culturable species between the samples. The second method of analysis will be the generation of 16S and whole genomic sequencing (WGS) data and subsequent bioinformatics analysis. The 16S sequencing will be used to determine if there is a change in the taxonomic profile between the samples after addition of the phytochemical and WGS will be used to determine if there is a change in antibiotic resistance gene composition in selected, phenotypically resistant bacterial strains.

2.2 Aims/objectives

The aim of this project is to determine how the addition of phytochemicals modulate the chicken microbiome and resistome, in a chicken caecum fermenter model. Assessment of the microbiome composition is the first priority as the antimicrobial effects of phytochemicals should be reflected in the microbiomes' species composition. Further the antimicrobial effects should be reflected in the composition of ARGs within the microbiome.

Hypothesis

1. The addition of phytochemicals during microbiome development results in significant changes in the bacterial species composition.
2. The addition of phytochemicals within an established microbiome will have different impacts on the species composition than hypothesis 1, but will still result in significant changes.
3. The addition of phytochemicals will result in significant alterations in the prevalence of ARGs and cross-resistance to antibiotics in both developing, and established microbiomes.

2.3 Expected research outcomes

The first outcome will explain how the addition of phytochemicals both prior and post the establishment of a stable microbiome within the caecum impacts the species composition. Samples will be taken from the model at different timepoints, subsequently DNA will be extracted from these samples and undergo 16S metagenomic profiling.

The second outcome will explore how addition of phytochemicals alter the prevalence of antimicrobial resistance genes within the chicken caecum. Samples from the models will be plated out onto differential and selective media to allow for the selection of specific bacterial species determined to be of interest from the 16S metagenomic profile (For example, if Enterobacteriaceae species increase it would be prudent to know if this is due to the Enterobacteriaceae antimicrobial resistance genes). The isolates of interest will also undergo phenotypic testing to determine their antimicrobial resistance profiles.

If time, and money permits. The samples from the caecum models will also undergo full metagenomic screening. This is however a very labour and cost intensive methodology and may provide far more data than is needed to answer the above questions.

This project will, collectively, increase the scientific understanding of the effects of phytochemicals as environmental drivers of antimicrobial resistance within the agricultural industry. Alongside this, the results will be compared to similar experiments conducted using traditional batch culture and in vivo chicken work. This will allow a direct comparison between the models and in vivo work which can be used be the wider scientific community to understand how accurately the model represents in vivo works and its potential benefits and shortfalls.

2.4 Methodology

This project will use a chicken caecum fermenter model for the analysis of the impact of phytochemicals on the microbiome and resistome of chickens. The model will be run using two different conditions against a control with no added phytochemical. Phytochemicals will be added at a concentration of 3-6g/kg, the concentration found in commercial phytochemical based growth promotors.

The first condition will analyse the impact of phytochemical addition after stabilisation of the microbiome. The model will be inoculated on day one, with phytochemicals added at day 3. Bacteria will be extracted on day 1, 3, 5 and 8. The second condition will analyse the impact of phytochemical addition prior to stabilisation of the microbiome. The model will be inoculated on day 1, and then immediately challenged. Bacteria will be extracted at day 1 (prior to phytochemical addition), 3 and 4.

The results for this project will be obtained through two methods. Firstly, 16S metagenomic profiling will determine if treatment with phytochemicals alters the speciation of the microbiome. Secondly bacterial extracts will be plated out onto selective media to allow for selection of specific species of interest from the metagenomic profiling. Selected culturable bacteria will undergo whole genome sequencing to determine if there has been a change within the resistome of the bacteria.

The project timeframe is split between the experimental models, and the bioinformatics analysis. The initial experiments at WUR will last for four weeks in July 2020. During this time bacterial samples will be taken from the model and DNA will be extracted to be used for 16s metagenomic profiling. The bioinformatics analysis will occur at Liverpool School of Tropical Medicine, where the remaining samples will undergo DNA extraction, the samples will be cultured on differential agar and specific bacterial isolates of interest will undergo WGS. Data analysis is expected to continue till December 2020.

2.5 Benefits to research

This project is part of my PhD investigations, currently at least one chapter. [redacted]

This project is also expected to result in publications focusing on: 1) the effect of phytochemicals on the microbiome and the resistome of chickens; and 2) the comparative effects of phytochemicals in the caecum model compared to in vivo studies and batch culture models.

Depending on the success of this project it is hoped the work may feed into a larger post-doctoral study, focusing on a wider range of phytochemicals across the agricultural space, and this is something I am considering pursuing via a fellowship route.

2.6 Benefits to professional development

I am a second-year PhD student at the Liverpool School of Tropical Medicine. Thus far my education has been very UK centric, having completed my undergraduate at the University of St Andrews, and my masters at the Liverpool School of Tropical Medicine. This project would be beneficial to my development in several ways.

Firstly, it would give me the opportunity to work in a completely different environment and gain an understanding of both the similarities and differences in scientific work in different countries. It would also give me the opportunity to work in an agriculture-based institute, which is completely different from my current focus within a Public Health-focused University. This opportunity will give me a more holistic insight into the interdisciplinary nature of scientific research, and the many systems that contribute to the current antimicrobial resistance crisis.

The fermenter system available at WUR is one of only a few in use globally, and experience on the system will be invaluable for future research. As the problem of antimicrobial resistance grows a more diverse and holistic (One Health) understanding of AMR and a complimentary scientific skillset, alongside clear evidence of collaborative working, will be invaluable for me as I move forward in my career, when applying for postdoctoral conditions.