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NON-TECHNICAL SUMMARY

The role of host genetics, intestinal structure and microbiome diversity in chicken gut health

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
    - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
    - (iii) Improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes

Key words

Broiler chickens, gut health, dysbiosis, selective breeding

Animal types	Life stages
Domestic fowl (Gallus gallus domesticus)	juvenile

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

Modern broiler chickens reared for meat commonly suffer from poor gut health. We aim to define early life physiological and microbiological markers that associate with good intestinal health and can be used in broiler breeding programmes or diagnostics to improve gut health and reduce reliance on antimicrobial drugs.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

More than 72 billion broiler chickens are produced in the world every year, most to provide meat for human consumption. Selection for performance (e.g. body weight gain, food conversion ratio) has been intense and in some examples unintended consequences have included predisposition to enteric dysbiosis. Enteric dysbiosis is a common condition where populations of bacteria and other microorganisms in the gut become unbalanced. Dysbiosis compromises broiler productivity and welfare, increasing the occurrence of disease, poor litter quality and lameness traits, as well as slaughterhouse condemnation resulting in poor welfare and wasted chicken production. Enteric dysbiosis is commonly prevented or controlled by routine application of antimicrobial prophylaxis, incurring a high requirement for antimicrobial use in chicken production. However, the use of antimicrobials in livestock production has been associated with selection for drug resistance in bacteria that might compromise human health. As chicken producers strive to reduce antimicrobial use alternatives are required, for example selective breeding to improve gut health. The well-established pyramid structure of broiler chicken production is highly amenable to selective breeding, with elite pedigree stock representing less than 0.0001% of chickens produced annually. Crossbreeding and amplification through great grand-/grand-/and parental generations produces ~4 million broiler chicks per pedigree female over a four-year cycle. Thus, improving gut health at the elite pedigree level will exert an enormous influence on farm-level broiler welfare and productivity, as well as reducing antimicrobial use. Here, we aim to identify a range of biological markers that can be used to identify individuals with good gut health from an early age to prioritise their use in selective breeding programmes.

What outputs do you think you will see at the end of this project?

The work will generate new data and knowledge about (1) fundamental biology of commercial broiler chickens, (2) the occurrence and consequences of poor chicken gut health, and (3) microbiological, physiological and transcriptomic markers that associate with good/bad gut health. These outputs are

also worthwhile in their own right because they contribute to understanding of the health of chickens and their microbiomes. The work will be published in peer-reviewed and industry journals, supporting the career development of the PhD students and early career postdoctoral scientists working on this project. The work is also essential for more targeted specific objectives:

Improved understanding of enteric dysbiosis in broiler chickens can be used to develop phenotypes associated with gut health that correlate with productivity and welfare. Such phenotypes can be used to improve commercial chicken lines by selective breeding and to highlight novel interventions.

Improved broiler chicken gut health is also expected to lower demand for antimicrobial intervention, reducing drug use in livestock production. Lower antimicrobial consumption will reduce selection for antimicrobial resistance in enteric and environmental microbial populations, and reduce antimicrobial flow into environments around chicken production systems.

Reducing the occurrence of ill health in chickens will lower the overall cost of poultry products, benefitting consumers as well as production and distribution networks.

The UK leads the world in breeding and production of broiler chickens through longstanding associations with companies such as Cobb-Vantress and Aviagen, both of whom have regional bases in the UK. Biomarkers associated with good gut health can be used to improve chicken lines and promote UK competitiveness.

Who or what will benefit from these outputs, and how?

The outputs are expected to provide benefits in the short, medium and long term.

Short term

Staff and students working on the project will receive training in simulated farm and laboratory level settings, including a range of protocols that can only be applied with live animals and can also be used to answer a variety of experimental questions beyond the remit of this work.

Medium term

Chicken breeding companies will benefit from improved knowledge of enteric dysbiosis and identification of biomarkers that can be applied to improve the health and welfare of pedigree chicken lines. Such improvements will also improve their national and international competitiveness.

The national and international scientific community will benefit from improved understanding of enteric dysbiosis.

Long term

Improved chicken lines produced by selection for biomarkers of intestinal health will cascade down the breeding pyramid, increasing health and welfare of breeding and, ultimately, commercial stock.

Farmers/poultry producers will benefit from healthier stock, improving performance and profitability.

Consumers will benefit from healthier, more cost-effective poultry products.

The general public will benefit from reduced antimicrobial use in poultry production, supporting reduced selection for resistance and lower risk of environmental contamination.

How will you look to maximise the outputs of this work?

All data produced from these studies will be published in Gold Open Access peer reviewed journals, as mandated and supported by the funding body (BBSRC). In addition to data, protocols and standards developed or applied will be described, providing resources and benchmarks for comparative studies. Data such as DNA or RNA sequences (e.g. microbiomes, host transcriptomes) will be submitted to open repositories, specifically the European Nucleotide Archive (ENA), linked to the DNA Data Bank of Japan (DDJB) and GenBank. Published studies will include results of null or unassociated measures. Results will be shared with peer audiences through national and international conferences (e.g. British and World Veterinary Poultry Association meetings).

Results and progress will also be reported in industry journals and magazines, as well as live events such as the Pig and Poultry Show, to ensure dissemination to relevant target audiences.

A series of collaborations will enhance outputs for the work. The work is supported by an industry partnering award (IPA), providing a direct link to industry. Both the applicant and partner also collaborate with researchers at national and international institutions that host world leading poultry biologists and geneticists.

Species and numbers of animals expected to be used

- Domestic fowl (Gallus gallus domesticus): We expect to use up to 400 pedigree broiler breeder chickens in data generation, up to 150 SPF Lohmann Valo chickens and 350 commercial broiler chickens in validation. We may also use up to 100 interleukin (IL)-10 knockout chickens to assess gut integrity in an inflammatory immune environment. Please refer to the specific protocol for associated power calculations.

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Chickens will be used throughout these studies, recognising that they are the target animal and not just a model. Enteric dysbiosis occurs naturally during production and will not be induced experimentally – we aim to identify markers that associate with good gut health. Clinical signs for enteric dysbiosis are difficult to define but include diarrhoea and lameness (bacterial chondronecrosis with osteomyelitis [BCO]), as well as non-specific signs such as lethargy, closed eyes and ruffled feathers.

A range of chicken types will be used, including (i) specific pathogen free chickens (Lohmann Valo) to permit accurate assessment of measures with minimal background variation, (ii) pedigree broiler

breeder lines, representing the target population, (iii) commercial broiler chickens, providing real-life examples for validation, and (iv) a new interleukin (IL)-10 knockout (KO) chicken line. Chicks will typically be used up to six weeks of age (maximum of eight weeks old), recognising that dysbiosis is primarily a problem during the early rapid phase of broiler growth.

Typically, what will be done to an animal used in your project?

A range of chickens will be used, including specific pathogen free (SPF) Lohmann Valo and IL-10 KO chickens (Experiment type A) and pedigree or hybrid commercial broiler chickens (Experiment type B).

Experiment type A: controlled conditions parameter setting

SPF Lohmann Valo or IL-10 KO chickens will be used to define natural variation in candidate biomarkers for enteric dysbiosis in a 'clean' system with reduced background variation (e.g. controlled absence of specific pathogens, restricted host genetic diversity). These chickens are not expected to experience clinical dysbiosis, but will provide data on natural variation in enteric structure and function, as well as all candidate biomarkers.

- Typical experiments will include receipt of chickens at one day or ~two weeks of age followed by a seven-day settling-in period in groups of two to ten individuals in wire floored cages.

- Chickens may be placed in single bird cages for up to four hours to permit collection of faecal material from specific individuals, after which they will be returned to their group cage. If a low volume of faecal material is required chickens may be cloacally swabbed as an alternative.

- Chickens may receive an oral dose of fluorescein isothiocyanate-dextran (FITC-d), followed by blood collection from a wing vein to assess FITC-d leakage from the gut. The process may be repeated on up to three occasions. Blood collected at the same time may also be used to assess a range of immune parameters including cytokine levels as well as bacteriaemia.

- Each study is expected to last a maximum of six weeks.

Experiment type B: commercial conditions data collection

Pedigree or commercial broiler chickens will be used to identify candidate biomarkers in the target population expected to experience natural dysbiosis. Broiler chickens are not being used as a model.

- Typical experiments will include receipt of chicks at one day of age (industry standard) followed by a seven-day settling period in groups of two or more individuals.

- Most studies will accommodate broiler chicks in floor pens, although wire-floored cages may be used for the first two weeks of life if directly comparing with Lohmann or IL-10 cohorts.

- Chickens will be reared under commercial conditions (e.g. stocking density, feed, lighting regime) to assess the natural occurrence of enteric dysbiosis and associated phenotypes.

- Chickens may be placed in single bird cages for up to four hours to permit collection of faecal material from specific individuals, after which they will be returned to their group pen or cage. If a low

volume of faecal material is required chickens may be cloacally swabbed as an alternative.

- Chickens may receive an oral dose of FITC-d, followed by blood collection from a wing vein to assess FITC-d leakage from the gut. The process may be repeated on up to three occasions. Blood collected at the same time may also be used to assess a range of immune parameters including cytokine levels as well as bacteriaemia.

- Each study is expected to last a maximum of eight weeks. During this period it is anticipated that 10-30% of pedigree individuals will experience enteric dysbiosis (3-15% for hybrid commercial broilers), a natural occurrence that is common to broiler chickens, although the level of occurrence may vary between chicken lines. Clinical signs for enteric dysbiosis are difficult to define but include diarrhoea and lameness (BCO), as well as non-specific signs such as lethargy, closed eyes and ruffled feathers. Chickens showing clinical signs of enteric dysbiosis will be removed from the study, culled, and sampled. Chickens showing clinical signs will not be retained.

What are the expected impacts and/or adverse effects for the animals during your project?

Enteric dysbiosis occurs naturally in up to 10-30% of commercial or pedigree broiler chickens when reared under commercial conditions (Experiment type B). Clinical signs are non-pathognomonic but include ruffling of feathers, paleness of comb and wattles, permanently closed eyes, wet droppings, diarrhoea and/or bloody faeces, or reluctance to move. Detection of dysbiosis is the objective of the project and all individuals will be removed and culled for sampling when two or more of the signs are observed, or if a single sign persists for >24hrs. Euthanasia will not be delayed for any experimental or procedural reason. Chickens used in experiments of type A will not be expected to experience clinical dysbiosis, but will contribute to definitions of enteric structure and function.

Impacts and adverse effects of the procedures described here are expected to be mild. Blood collection from the wing vein can result in a localised haematoma but these are tolerated well. The risk is far lower than sampling from the jugular vein and consequences usually resolve within 24 hours (Livingstone, 2020). Oral inoculation is straightforward in chickens and very well tolerated.

Livingstone, M., CPD article: How to perform venipuncture in avian patients. Companion Animal, 2020. 25(10).

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Severities of the procedures carried out in this project are expected to be mild.

The natural occurrence of enteric dysbiosis is anticipated in up to 10-30% of individuals (current commercial expectations, data provided by the commercial partner), but will not be induced by any experimental procedure. SPF Lohmann and IL-10 KO chickens are not expected to experience enteric dysbiosis.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Animals are required for this project in the absence of a suitable in vitro/ex vivo alternative. Enteric dysbiosis occurs naturally during broiler production, primarily between three and five weeks of age. We aim to collect a range of data from chickens prior to the occurrence of dysbiosis that can be tested as predictors for good gut health.

Which non-animal alternatives did you consider for use in this project?

Non-animal alternatives are not currently available for enteric dysbiosis. Compartmental models such as fermenters (gut lumen models), organoids and tissue explants represent incomplete systems and are not currently fit for purpose. The topic has been reviewed during preparation of this application (e.g. targeted searches of the published literature via PubMed, Web of Science and Google Scholar). Progress with explants and organoids has been made in recent years (e.g. studies at the Roslin Research Institute; doi: 10.1038/s42003-021-01901-z), but lack the ability to replicate interactions between host, environment and microbiome.

Why were they not suitable?

Non-animal alternatives cannot be used to re-create the complex interaction between host, environment and microbiome required to assess enteric dysbiosis.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The numbers requested have been estimated based on the expected occurrence of dysbiosis under production conditions and the numbers of individuals required in each phenotype group. Dysbiosis is anticipated in 10-30% of pedigree chickens (data provided by the commercial partner, supplier of the broiler chickens), and 3-15% of commercial broiler chickens. Our studies of enteric microbiome

diversity indicate a requirement for at least ten individuals per phenotype to reliably identify microbiome type (termed 'enterotype').

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

We intend to use four different types of chicken during these studies to minimise biological variation and improve statistical power. First, using a known pedigree broiler breeder line provides reproducible appearance of enteric dysbiosis in 10-30% of individuals during normal production. The occurrence of dysbiosis is less predictable in most other chicken types. These pedigree birds are also defined by lower genetic diversity than hybrid commercial broiler chickens, improving reproducibility and permitting smaller group sizes (i.e. the top versus the bottom of the broiler breeding pyramid). SPF Lohmann Valo chickens provide additional control of microbiome variation with a similar genetically homogeneous background. Commercial broiler chickens will be used for validation, while IL-10 knockout chickens may be used to define the importance of the anti-inflammatory immune response.

We will use oral inoculation of fluorescein isothiocyanate-dextran (FITC-d) and subsequent measurement in serum to assess gut integrity and leakage, permitting repeated sampling from individual animals without a requirement for slaughter. The procedure is mild and reduces the number of chickens required.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

The studies proposed here have been based on current commercial knowledge and existing pilot data collected from broiler breeder populations in the field, where we have opportunistically sampled birds culled for management purposes. We have also established considerable background data defining variation in measures such as microbiome diversity, transcription profiles and bacteriaemia from previous studies in the UK and overseas that can be used in power calculations (as outlined in protocol 1). Samples collected from these studies will be blinded for laboratory analyses. Tissues and data will be shared with other projects within the group and made available to others within the wider College community.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Industry data indicate that up to 10-30% of pedigree and commercial broiler chickens will experience enteric dysbiosis by five weeks of age when reared under standard commercial conditions in the

absence of antimicrobial prophylaxis. For this reason, we will not include any experimental procedure to induce dysbiosis – we will re-create standard farm conditions. The procedures listed here (oral inoculation, blood sampling, and cloacal swabbing) are well established and selected to minimise the need for invasive procedures. For example, final blood sampling will be undertaken immediately post-mortem rather than from live birds to minimise the number of procedures per individual.

Why can't you use animals that are less sentient?

The study of enteric dysbiosis in chickens cannot be accurately replicated in any other less sentient animal.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Chickens will be habituated to experimental staff from arrival, including routine 'pen walk throughs' that will be used to detect chickens experiencing enteric dysbiosis.

Based on experience gained in recent studies with commercial broiler chickens it is clear that enteric dysbiosis is most likely to occur between three and five weeks of age. We will increase monitoring during this period.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We will follow ARRIVE guidelines for experimental reporting in study design to ensure the most relevant and transparent experimental design. We will also apply community consensus guidelines for reporting (e.g. adapting the human microbiome STORMS checklist to poultry; Mirzayi et al., 2021) to study design to ensure all sampling is fit for purpose.

We will follow expert guidance on husbandry from our commercial partner, ensuring that best practices are always followed.

Mirzayi C et al. (2021) Reporting guidelines for human microbiome research: the STORMS checklist. Nat Med 27(11):1885-1892.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

The applicant and all team members who will work on the project regularly interact with the NC3Rs, checking the website regularly and attending seminars and webinars when they occur. The host organisation is also very active in dissemination of 3Rs relevant news, providing training and updates via a newsletter, emails and online notifications.