Control of avian pathogenic *Escherichia coli* by non-antibiotic compounds inducing trained immunity of chicken macrophages

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Progress summary

Bacterial diseases can have a devastating impact on poultry health, welfare and productivity in all poultry rearing systems. Avian Pathogenic *Esherichia coli* (APEC) causes avian colibacillosis in all bird species, leading to widespread financial and animal health and welfare issues. Avian colibacillosis results in increased morbidity and mortality, diminished egg production, and reduced hatchability (Kabir *et al.*, 2017). Antibiotics have historically been widely used to treat or prevent bacterial infections, in addition to promoting growth in poultry. However, this usage has contributed to the selection of antibiotic-resistant bacteria, which can cause serious health issues in both humans and animals (Sargeant *et al.*, 2019). Alternative strategies such as vaccination are also used to control bacterial diseases in poultry, but the highly diverse nature of APEC can lead to reduced effectiveness of such approaches (Sadeyen *et al.*, 2015; Gharib *et al.*, 2017; Abd El-Mawgoud *et al.*, 2020; Koutsianos *et al.*, 2020; Paudel *et al.*, 2024). Therefore, it is crucial for the poultry industry to take proactive steps to reduce the use of antibiotics and seek alternative strategies for controlling and treating bacterial infections.

One suggested alternative strategy is the generation of immunological memory in the innate immune system (Adams *et al.*, 2023). Immunological memory refers to the ability of an immune cell to recall a previous encounter with a microbial invader and use this information to speed up and improve their response. This is what is seen within common vaccines, where an inactivated or modified version of the pathogen is introduced into the target host (human/animal) so this immunological memory can be generated. However, vaccines typically target the highly specific adaptive immune system, so even minor changes in the bacteria may prevent recognition, thus allowing the bacterial pathogen to cause disease. In contrast, the innate immune system has no such specificity, allowing it to respond rapidly to a wide number

of pathogens, with the generation of innate immunological memory having been observed to provide protection against a broad range of bacterial agents (Adams *et al.*, 2023). Immunological memory in the innate immune system can take several distinct forms. Immune priming involves the activation of innate immune cells by an immunomodulatory compound during bacterial challenge, leading to enhanced responses and subsequent protection from infection. Additionally, immunological memory can take the form of trained immunity which is highly similar to traditional vaccine approaches. Activation of the innate immune system with an immunological agent leads to changes within the immune cells which persist despite the removal of the activating compound, leading to enhanced responses to future bacterial challenges (Divangahi *et al.*, 2021). Immunological memory has been demonstrated to be effective within animals, with trained immunity generated by injection of parts of a fungal cell being used to prevent mice from being infected by several bacteria including *E. coli (Ciarlo et al*., 2020). With the highly diverse nature of APEC proving a challenge for the development of effective vaccines, the targeting of immunological memory shows promise as a viable alternative.

This PhD project aimed to develop novel non-antibiotic management strategies for APEC, through the targeting of immunological memory. Initially, interactions between distinct APEC genotypes and avian innate immune cells were characterised using cell culture and insect infection models. This revealed heterologous phenotypic interactions with avian epithelial and macrophage cell lines, as well as virulence capacity in *Galleria mellonella* (Greater wax moth larvae) between APEC genotypes. This is suggestive of distinct phenotypes and cellular interactions within the APEC pathotype and highlights the potential viability of non-specific immunomodulation strategies in the control of APEC in poultry and informed the selection of potential agents for further investigation.

While effective at protecting against infection, approaches such as trained immunity have been linked with inflammation and immunopathology, potentially leading to animal health and welfare issues in poultry and reduced productivity (Funes *et al.*, 2022). Therefore, it is important to develop approaches with the potential to mitigate inflammatory responses. Thus, the short chain fatty acid sodium butyrate was selected for investigation as it has been previously demonstrated to enhance chicken macrophage antimicrobial activity, while also limiting inflammation within the chicken gut (Panda *et al.*, 2009; Sunkara *et al.*, 2011; Ahsan *et al.*, 2016). Priming with sodium butyrate was demonstrated to enhance bacterial clearance

as a result of enhanced reactive oxygen species (ROS) production, autophagic intracellular degradation, in addition to the inhibition of the mechanistic target of rapamycin (mTOR) within chicken macrophages. The ability of sodium butyrate to induce trained immunity, was also examined for the first time, with classical responses after activation observed following training with sodium butyrate. Training was also demonstrated to enhance chicken macrophage antimicrobial activity against a range of bacterial pathogens, with this antimicrobial activity shown to be dependent on increased ROS production and autophagic function, mirroring priming of chicken macrophages. Collectively, the study of both priming and training as immunomodulation strategies, highlighted the potential utility of non-antibiotic immunomodulation of chicken macrophages in the prevention of bacterial disease in poultry.

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