IMPACT OF PHAGE THERAPY AND LAIRAGE ON SALMONELLA AND CECAL MICROBIOTA OF BROILERS DURING IN VITRO INCUBATION

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I. INTRODUCTION

Pre-harvest food safety interventions are designed to reduce the load of foodborne pathogens entering a processing facility. Routinely, animals are subject to a period of feed withdrawal prior to harvest to aid in ease of processing and reduce risk of contamination. In recent years more targeted interventions, such as phage therapy, have been developed but more investigation is required to determine their efficacy and optimal time of application during the growout period prior to processing [1,2]. Therefore, the objectives of this study were to evaluate the impact of phage therapy on inoculated *Salmonella* populations during *in vitro* incubation of broiler ceca and characterise the impact of treatment and lairage on the cecal microbial community.

II. MATERIALS AND METHODS

One day post-hatch, broilers were obtained from a local hatchery in Wisconsin and placed on floor pens at the UW-Madison Poultry Research Laboratory where they were provided water and a commercial corn-soybean diet *ad libitum*. At 63 d of age, Ross 708 broilers were subjected to either no feed withdrawal (NFW) or an 8 h feed withdrawal (FW; N=20, n=10) before being humanely euthanized and cecal contents collected. Cecal contents were transferred to an anaerobic chamber, diluted (1:3000) in anaerobic dilution solution, and aliquoted (20 mL) into the following *in vitro* treatments for a 2 x 4 factorial treatment design: (1) NFW Ceca only, (2) FW Ceca only, (3) NFW Phage, (4) FW Phage, (5) NFW *Salmonella*, (6) FW *Salmonella*, (7) NFW *Salmonella* + Phage, (8) FW *Salmonella* + Phage. Where applicable, *in vitro* treatments were inoculated with an overnight culture of a nalidixic acid resistant strain of *S*. Typhimurium at $1x10^7$ CFU/mL and $1x10^6$ pfu/mL *Salmonella* Bacteriophage P22 (MOI 0.1). Cultures were incubated anaerobically at 37°C and sampled at time 0, 3, and 6 h for *Salmonella* enumeration, via dot plating on XLT-4, and 16S rRNA gene sequencing. Genomic DNA was extracted using Qiagen DNeasy® Blood and Tissue kit and the 16S rDNA was sequenced using an Illumina MiSeq. Pathogen data was log transformed and analyzed in JMP15 using a mixed model with repeated measures and means separated by Tukey's Protected HSD ($P \leq 0.05$). Sequences were filtered and aligned using the QIIME2-2023.2 pipeline. Statistical significance of α-diversity and β-diversity metrics were determined using ANOVA and ADONIS, respectively, and an analysis of composition of microbiomes (ANCOM) was performed to distinguish between differentially abundant taxa. Data was considered significant at $P \leq 0.05$ and pairwise differences at $Q \leq 0.05$.

III. RESULTS AND DISCUSSION

There was a significant effect of *in vitro* treatment between the *Salmonella* and *Salmonella* + Phage groups ($P < 0.05$). Reductions in overall *Salmonella* population were not observed; however, phage therapy did result in a lower level of *Salmonella* over time compared to other *in vitro* treatments. The greatest impacts on *Salmonella* were observed between 0 and 3 h of incubation, suggesting the inactivation potential of P22 is greatest immediately after inoculation. Feed withdrawal did not have a significant effect on the level of *Salmonella* with or without phage therapy ($P>0.05$). Species richness (Observed features) was heavily

impacted by *in vitro* treatment, with the Ceca only and Phage groups demonstrating a higher degree of community richness (P<0.05). Species evenness (Pielou Evenness) was impacted by feed withdrawal, *in vitro* treatment, incubation time, and the interaction between all three (P<0.05). A greater evenness was observed among the FW birds in addition to the Ceca only and Phage *in vitro* treatments. Incubation time had an inverse impact of the *in vitro* treatments, with the evenness of Ceca only and Phage reducing over time and the evenness of *Salmonella* and *Salmonella* + Phage increasing over time. β-diversity metrics (Bray-Curtis and Weighted UniFrac) were impacted by feed withdrawal, *in vitro* treatment, and incubation time (P<0.05). Unsurprisingly, the treatments that demonstrated the most similar microbial communities were either not inoculated with *Salmonella* (Ceca only and Phage) or inoculated with *Salmonella* (*Salmonella* and *Salmonella* + Phage; Figure 1). The microbial composition of the ceca without artificial inoculation at 0 h mostly consisted of the phyla *Firmicutes*, *Cyanobacter*, *Campilobacterota*, and *Bacteroidota*. Slight visual differences were observed between the FW and NFW ceca, with a greater relative abundance of *Cynobacteria* and *Bacteroidota* observed in the FW birds (9.02% vs. 6.62% and 15.91% vs. 12.11%, respectively). However, the only taxonomic groups found to be differentially abundant were the phyla *Campilobacterota* which was found in a greater abundance among NFW birds (W=9; 10.82% vs. 2.54%; P<0.05), and the family *Enterobacteriaceae* which was found to be more abundant in FW birds (W=27; P<0.05).

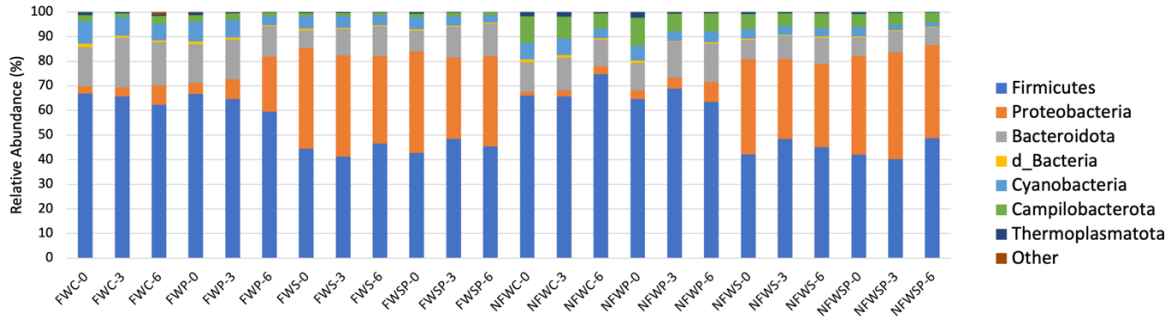

Figure 1: Taxonomic bar plot of *in vitro* cecal microbiota during incubation at the phylum level.

IV. CONCLUSION

Overall, phage therapy may be a viable pre-harvest *Salmonella* intervention strategy, but it is clear that a variety of factors including treatment duration and feed withdrawal status can impact antimicrobial efficacy and overall microbial composition.

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