What is the poultry microbiome and why should I care about it?

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Introduction.

Until recently, gut health and development in poultry has been routinely managed through the use of low-dose levels of antibiotics in feed to prevent diseases, improve overall flock consistency, and enhance performance. However, worldwide efforts are underway to reduce and/or eliminate antibiotic use in animal agriculture. The elimination of this valuable management tool for use by farmers will leave a critical void that needs to be filled. We must understand the mechanisms by which bacterial succession occurs in the avian gastrointestinal tract in coordination with the host if we wish to identify antibiotic-free ways to modulate the microbiome to prevent disease and improve bird performance. Work in our laboratory has focused on understanding the succession of bacteria in the gastrointestinal tract of poultry. Defining this baseline is critical towards assessing the impact of products on the microbiome.

Bacterial species that correlate with weight gain in turkeys.

We previously performed longitudinal and cross-sectional sampling of fourteen flocks on seven farms. Research flocks were also sampled in this initiative. The entirety of the bacterial microbiome in these flocks was examined related to average daily weight gain. We demonstrated that there is a clear succession of bacterial species in the turkey ileum over the course of 12 weeks of age, involving the same dominant species previously associated with Heavy vs. Light flocks (Fig. 1). Comparing research versus commercial flocks, it was clear that the timing of bacterial succession occurred earlier in research flocks than it did in commercial flocks, where research flocks had higher rates of weight gain than commercial flocks. We were able to identify three distinct phases of maturity of the turkey ileum microbiome (Fig. 1), and biomarkers of microbiome succession delineating these phases. Overall, the bacterial microbiome composition in turkeys is highly predictable, and the timing of succession correlates with flock outcome in terms of performance.

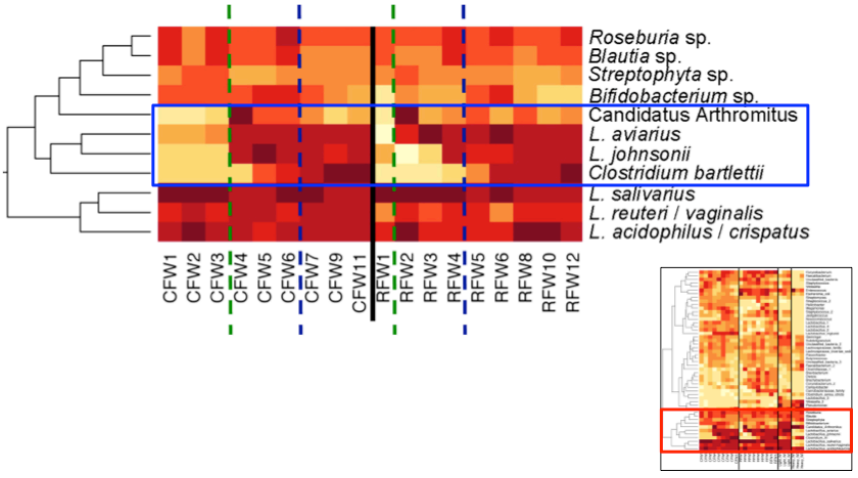
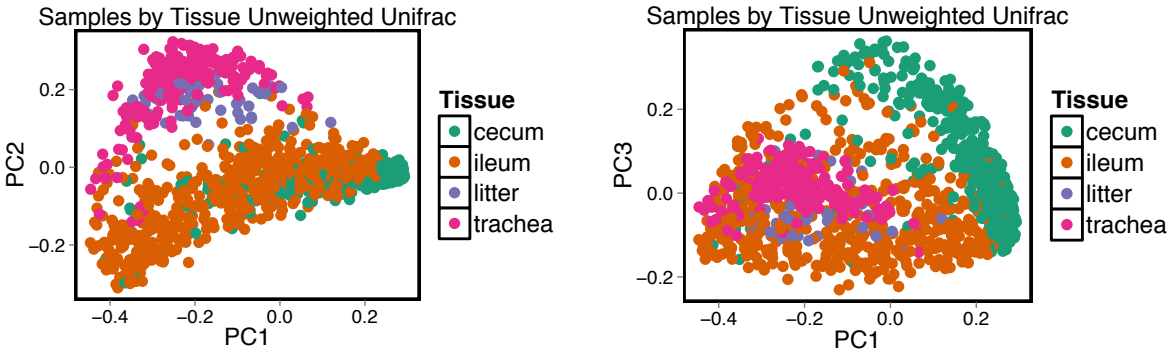


Figure 1 (left). Dominant bacteria in the turkey microbiome. Outlined in blue are species that were identified as biomarkers of enhanced flock performance. Dashed lines indicate phases of microbiome maturation.

How does the broiler microbiome compare to the turkey microbiome?
We have also performed sampling on broilers to determine their baseline bacterial microbiome and compare it with that of turkeys. Four commercial broiler flocks were followed for two successive flocks cycles. At days 0, 7, 14, 21, 28, 35, and 42, ten birds per flock and time point were euthanized and ileum, ceca, trachea, and litter were collected. In total, 1,558 samples were analyzed. Broiler samples clearly separated by sample type, with litter and trachea samples overlapping considerably, while cecum and ileum samples each separated uniquely (Fig. 2). This suggests that the tracheal bacterial microbiome is reflective of the barn environment, which is logical since birds are continually breathing air containing litter particles.

Figure 2 (below). Principal coordinate analysis plots of broiler samples by sample type. On the left, the two most informative dimensions are depicted, while on the right, the second and third most informative dimensions are depicted. Each dot represents the entire bacterial microbiome of a sample. Distances depict relative community similarity, with closer dots representing communities that are more similar than farther dots.



We then compared the bacterial microbiomes of chickens and turkeys with the hypothesis that they should be closely related but distinct. At first glance, chicken and turkey samples overlapped considerably with no apparent discrimination (Fig. 3). However, as these samples were broken down by tissue, it became apparent that there are subtle and predictable differences between the turkey and chicken bacterial microbiomes. In short, turkey and chicken microbiomes contain the same dominant species, but differ subtly in their rare microbiome making them distinct. This further suggests to us that there is indeed host adaptation of the microbiome even between chickens and turkeys, emphasizing the need to consider them uniquely when considering probiotic approaches.

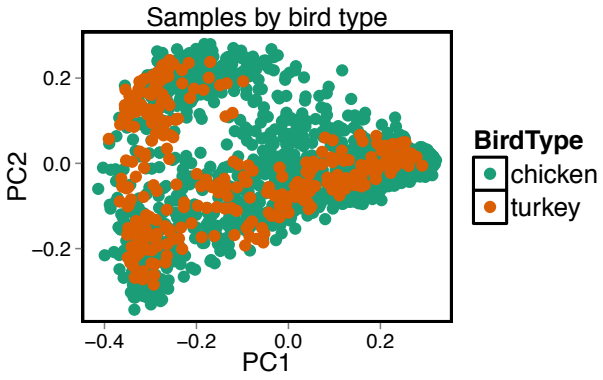
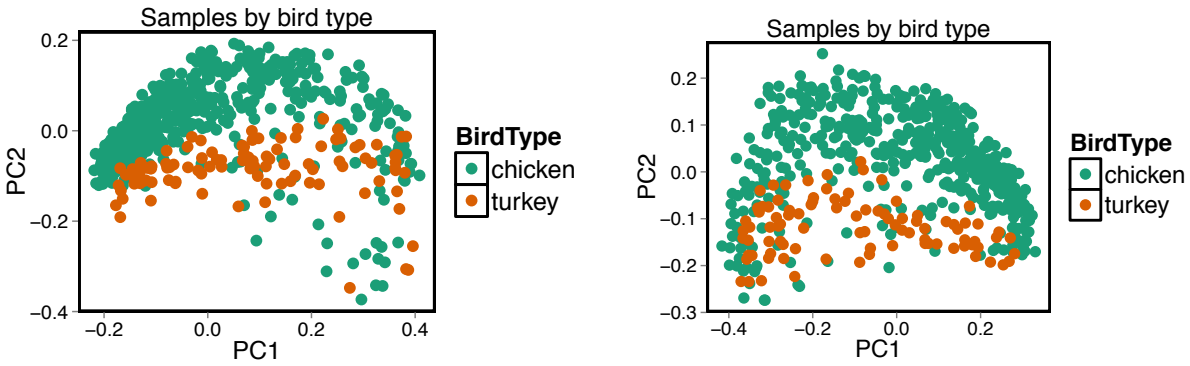


Figure 3 (left). Principal coordinate analysis plot of chicken versus turkey samples including all sample types (ileum, cecum, trachea, litter).

Figure 4 (below). Principal coordinate analysis plots of the bacterial microbiomes of chickens versus turkeys. On the left are cecum samples, and on the right are ileum samples. While chicken and turkey samples share the same dominant bacterial species and trend in the same direction over time, the rare microbiome makes them unique from one another.



Can the poultry microbiome be modulated?

An important question is if products used for gut health actually modulate the microbiome in turkeys, and the turkey host itself. Caged performance experiments were conducted using turkey poults, with eight cage replicates per treatment group and ten birds per cage (n=400). Treatments included a negative control, a GroGel carrier control, continuous subtherapeutic bacitracin methylenedisalicylate administration (50 g/ton) in feed, a commercial probiotic (FM-B11) administered daily via GroGel carrier, and an experimental 10-strain probiotic derived from turkey gastrointestinal bacteria administered daily via GroGel carrier. Tissues from birds were collected at days 3, 6, and 13 of age (spleen, ileum, cecum, trachea) and used for assessments of host gene expression via RNA-Seq, and bacterial communities via amplicon sequencing. Body weights and feed consumption were measured throughout the experiment.

Early significant differences were observed in average daily weight gain between treatment groups, with the turkey-specific probiotic resulting in the best performance measurements throughout the experiment (Fig. 5). Both antibiotic and probiotic

administration shifted the bacterial communities in the gut, with modulations from probiotic treatments peaking at day 6 of age and modulations from BMD treatment continuing throughout the trial (Fig. 6). RNA-Seq revealed the largest effects on the turkey host gene expression at the gut mucosal level, with BMD administration impacting 1,093 genes at day 6, while the turkey-specific and FM-B11 probiotics impacted 323 and 4 genes, respectively. Overall, this work identifies key mechanistic differences between antibiotic and probiotic treatments relative to the host-microbiome continuum, and confirms the ability of alternative products to at least temporarily modulate the poultry gut microbiome.

Figure 5 (below). Weights in grams of control versus treatment groups at days 6 and 13 of an experiment assessing the impact of low-dose antibiotics versus probiotics in turkeys.

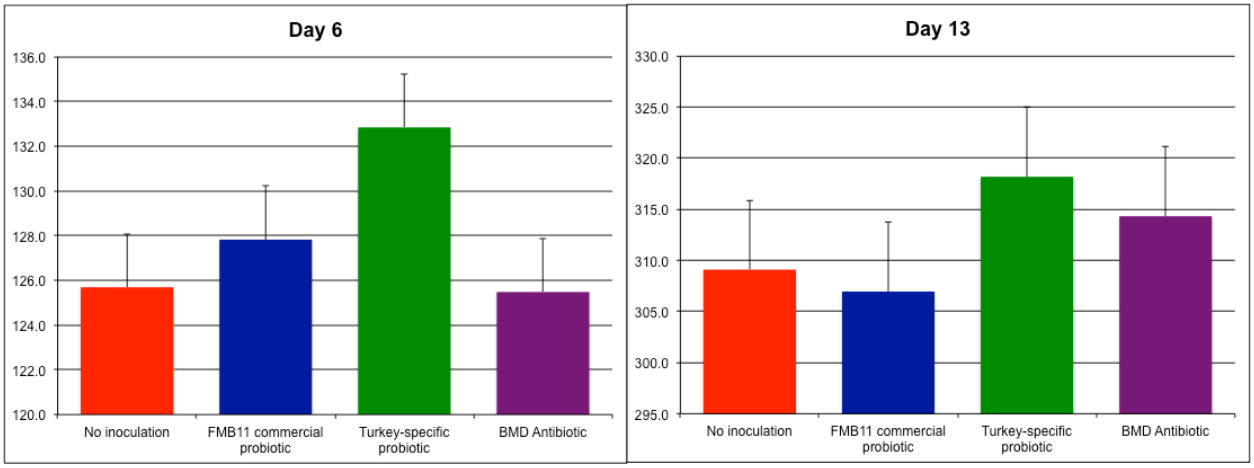


Figure 6 (below). Principal coordinate analysis plots on the bacterial microbiome of control versus antibiotic and probiotic treatments. All treatments impacted the microbiome at day 6, while BMD impacted the microbiome throughout the experiment.