College of Veterinary Medicine

University of Minnesota

UNIVERSITY OF MINNESOTA RESEARCH PROJECT:

TESTING THE ASSIST NPS PROGRAM (BROILERS)

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University of Minnesota | Department of Veterinary and Biomedical Sciences | College of Veterinary Medicine

Assist
Natural Products and Services LLC

UNIVERSITY OF MINNESOTA RESEARCH PROJECT:

TESTING THE ASSIST NPS PROGRAM (BROILERS)

A research study was conducted in the laboratory of Dr. Timothy Johnson at the University of Minnesota to test the impact of the ASSIST programmed approach on broiler performance and the broiler gastrointestinal microbiota.

MATERIALS AND METHODS

EXPERIMENTAL DESIGN

A total of 200 broilers were used in this study. Birds were acquired from a commercial source at day-of-hatch and randomly distributed into three dietary treatments with replicate pens (50 birds per group). The groups included: 1) two groups of birds receiving a standard corn-soy diet, each used as control groups on separate occasions for comparison with treatment groups; 2) one group of birds in two replicate pens receiving a standard corn-soy diet supplemented with Quickstart plus mixed in feed (Assist Natural Products and Services, LLC, Lena, IL), Pro-Oxine supplemented continuously in drinking water (BioCide International, Norman, OK), and pre-treatment of litter shavings with Relentless Plus (Assist Natural Products and Services, LLC, Lena, IL), all according to manufacturer's instructions; and 3) one group of birds in replicate pens receiving a standard corn-soy diet, Quickstart plus supplemented continuously in drinking water (Assist Natural Products and Services, LLC, Lena, IL), Pro-Oxine supplemented continuously in drinking water (BioCide International, Norman, OK), and pre-treatment of litter shavings with Relentless Plus (Assist Natural Products and Services, LLC, Lena, IL), all according to manufacturer's instructions. Birds were followed from hatch through 42 days of age. The protocol was approved by the Institutional Animal Care and Use Committee at the University of Minnesota, protocol 1309-30946A.

SAMPLING

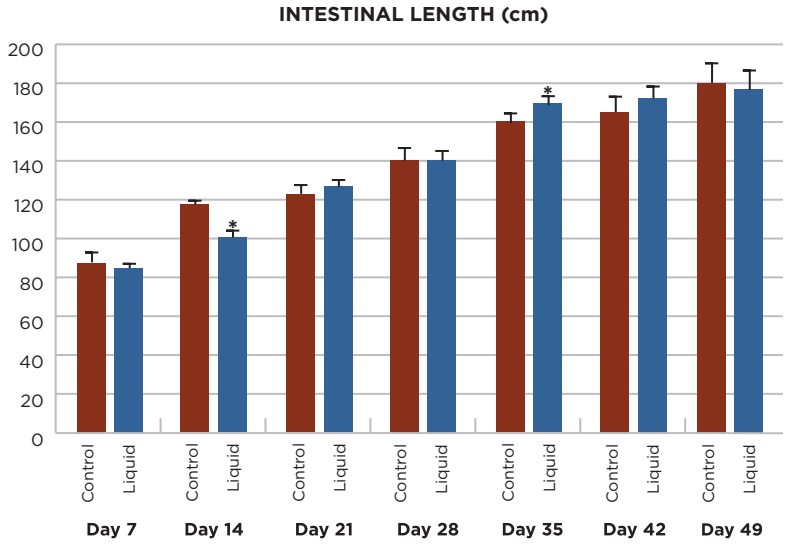
At weekly timepoints from 1-6 weeks of age, 6-8 birds per pen were randomly selected and euthanized. Staff were blinded to the experimental groups during bird postings. At euthanization, the following parameters were recorded: total bird weight, total intestinal weight, total intestinal length, cecal score, and ileum length. Ilea from each bird were aseptically collected and processed for 16S rRNA microbiome profiling, as described below. From birds at 21 days of age, approximately 1 cm of the distal jejunum was collected from each bird and fixed in 10% formalin and embedded in paraffin. Villi heights and crypt depths were examined using formalin-fixed and paraffin-embedded samples under a light microscope at 100x, and expressed as an average of ten fields per sample.

16S rRNA BACTERIAL MICROBIOME PROFILING

DNA was extracted using a bead-beating procedure and the QIAmp® DNA Stool Kit (Qiagen, Valencia, CA) as previously described [1]. The V1-V3 hypervariable regions of the 16S rRNA gene were amplified in 25ul reactions containing 1X PCR buffer (containing 1.8 mM MgCl2), 0.2 mM each dNTP (Promega, Madison, WI), 0.4QM each primer (Integrated DNA Technologies, Coralville, IA), 1.25 U FastStart High Fidelity Taq polymerase (Roche, Basel, Switzerland). Primers were designed for Illumina barcoding and sequencing as previously described [2]. Each forward and reverse primer contained a sample-specific sequence barcode. The PCR conditions used were an initial denaturation of 95°C for 2 min, followed by 25 cycles of 95°C for 30 sec, 60°C for 30 sec and 72°C for 30 sec; the amplification was completed with a final extension of 72°C for 7 min. The PCR product was excised from a 1.5% gel and purified using the QIAquick Gel Extraction Kit following manufacturer's instructions (Qiagen). Sample DNA quality and quantity were assessed on a Bioanalyzer 2100 (Agilent, Palo Alto, CA) using a DNA-1000 lab chip. Sequencing was performed at the University of Minnesota using Illumina MiSeq paired-end 2X300 bp technology.

DATA ANALYSIS

Following sequencing, sorting by barcode was performed to generate fastq files for each sample, which were quality-assessed and filtered prior to analysis. A de novo operational taxonomic unit (OTU) picking approach was used in QIIME [3] using uclust [4]. Potential chimeras were removed using ChimeraSlayer [4]. Approximately-maximum-likelihood phylogenetic trees were constructed using FastTree [5]. QIIME was also used for assessments of alpha diversity, beta diversity using Unifrac [6], and phylogenetic classifications using the RDP database [7,8]. Differential abundances of OTUs and other phylogenetic classifications were identified using METASTATS [9]. Construction of heatmaps was performed using the R statistical software [10].

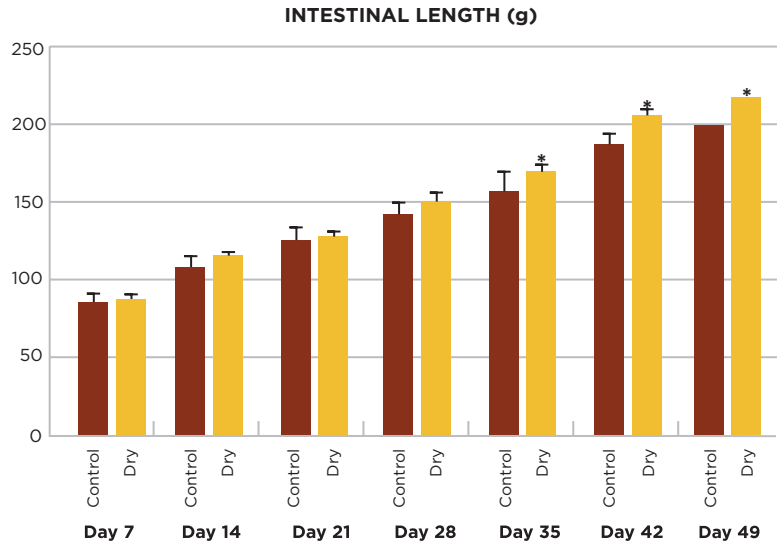


RESULTS

1

Intestinal lengths of birds in Control versus in-water Assist-treated groups.
Sampling of intestinal length was performed by measuring length in cm from the proximal end of the small intestine through the ileocecal junction (Figures 1 and 2; "*" denotes P<0.05).

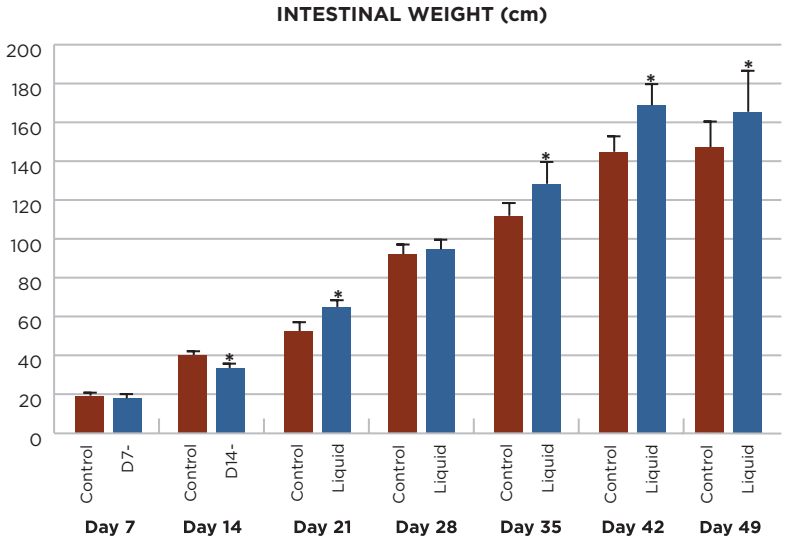
Control **Liquid**



2

Intestinal lengths of birds in Control versus in-feed Assist-treated groups.
No patterns in significant differences were observed in intestinal lengths at any of the timepoints between control and Assist-treated groups.

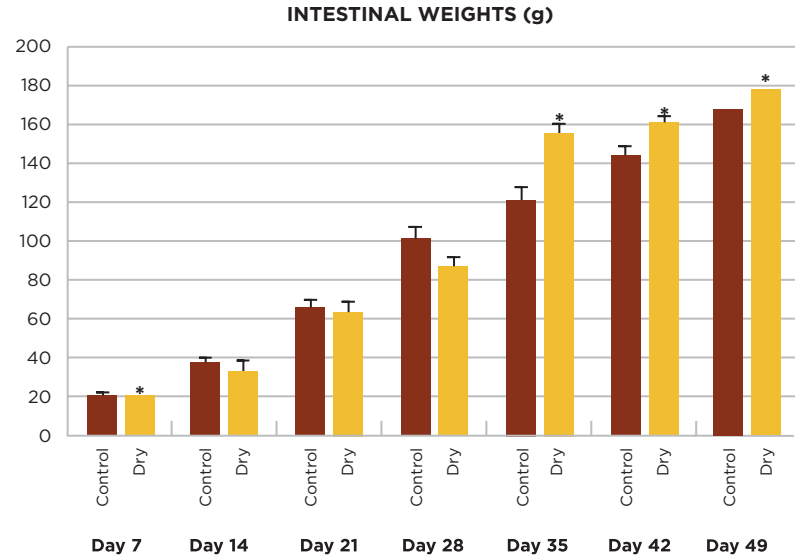
Control **Dry**



3

Intestinal weights of birds in Control versus in-water Assist-treated groups.
Similarly, intestinal weights were assessed at euthanization by weighing the same section of intestine used for intestinal lengths above (Figures 3 and 4; "*" denotes P<0.05).

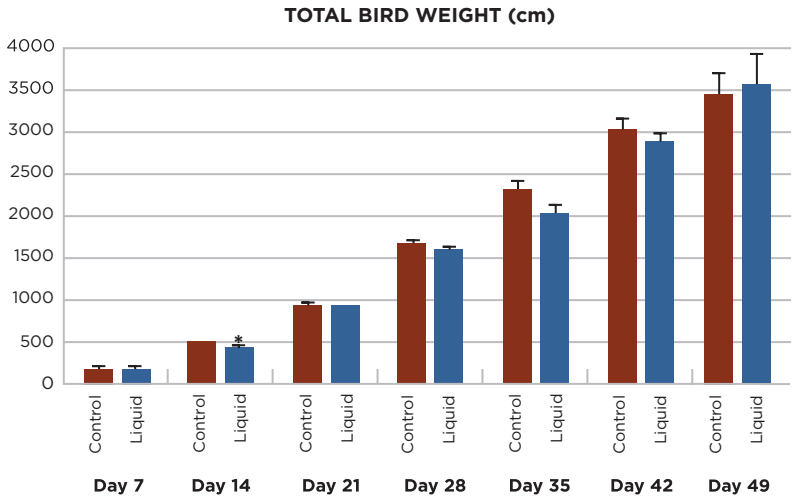
Control **Liquid**



4

Intestinal weights of birds in Control versus in-feed Assist-treated groups. In this analysis, intestinal weights in Assist-treated birds were significantly higher than control groups at several timepoints with in-feed treatment and in-water treatment, particularly at later timepoints.

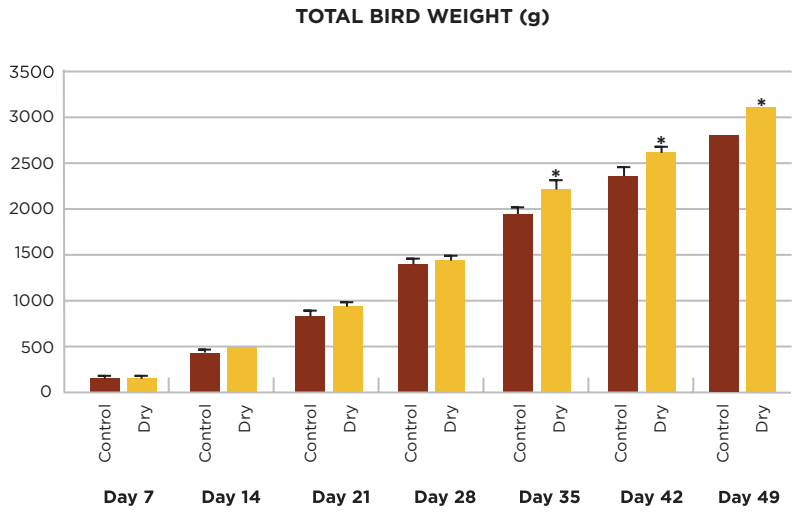
Control **Dry**



5

Total bird weights of Control versus in-water Assist-treated groups. Total bird weights were also measured at euthanization in the control versus Assist-treated groups at each timepoint (Figures 5 and 6; "*" denotes $P<0.05$). In general, in-water Assist treated birds were not significantly heavier than control groups.

Control **Liquid**



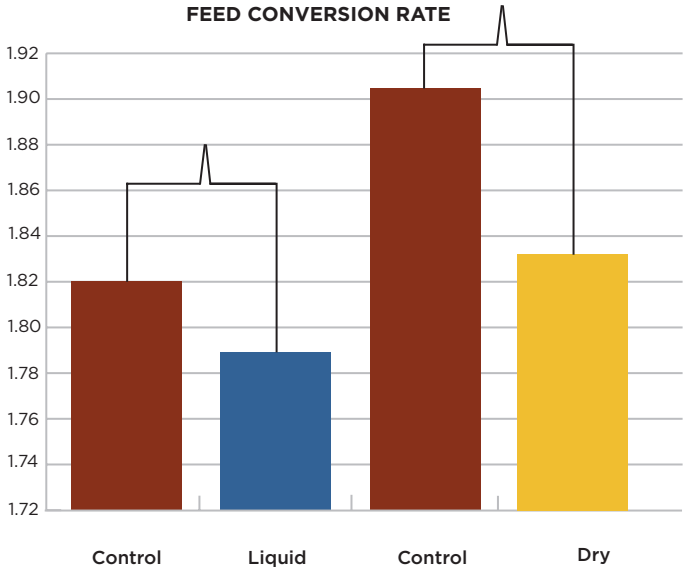
6

Total bird weights of Control versus in-feed Assist-treated groups. In contrast, in-feed Assist treated birds produced significantly heavier birds at 42 days of age than control groups. Ileum lengths and counts of total *Escherichia coli* were also performed (data not shown). No significant differences were observed overall between control and Assist-treated groups for either assessment.

Control **Dry**

FEED CONVERSION RATES

Feed conversion rates were also calculated between control and treatment groups by dividing total feed consumed by total bird weights at euthanization (Figure 7). In both the in-feed and in-water Assist treatments, FCR was better (-0.03 for in-water treatment, and -0.07 for in-feed treatment).



7

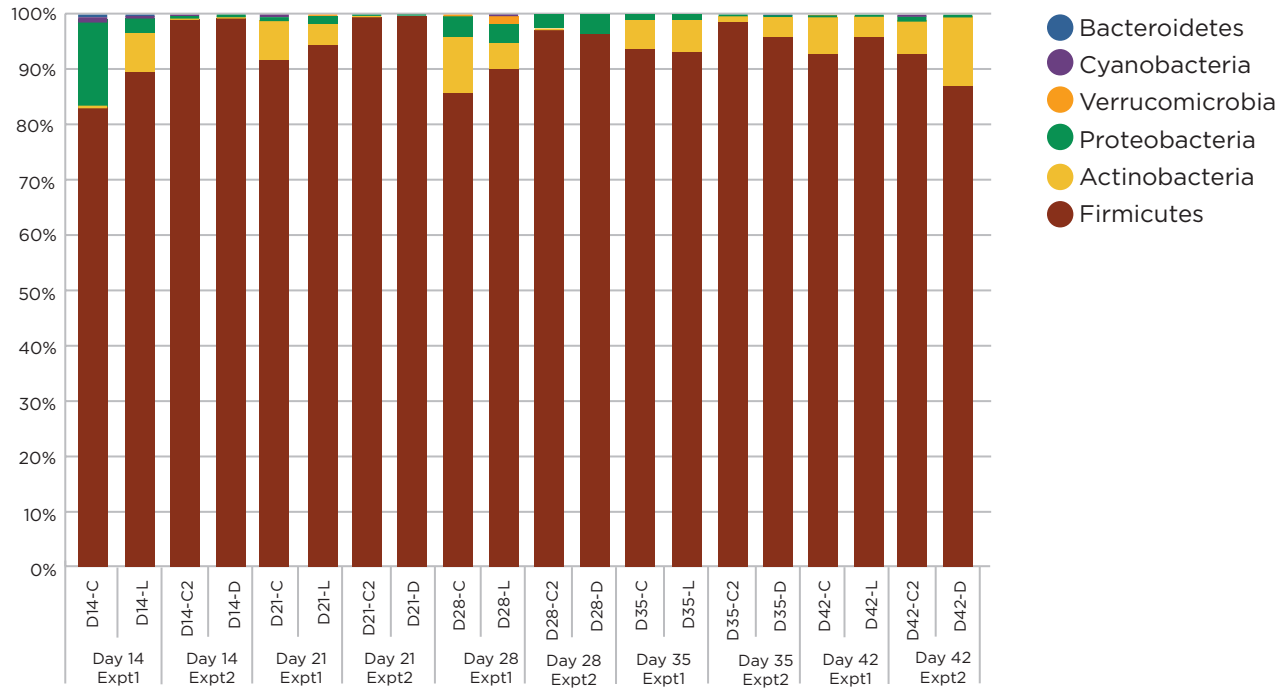
Feed conversion rates for Assist treatment groups compared to their respective control groups.

PHYLUM-LEVEL AND GENUS-LEVEL BACTERIA

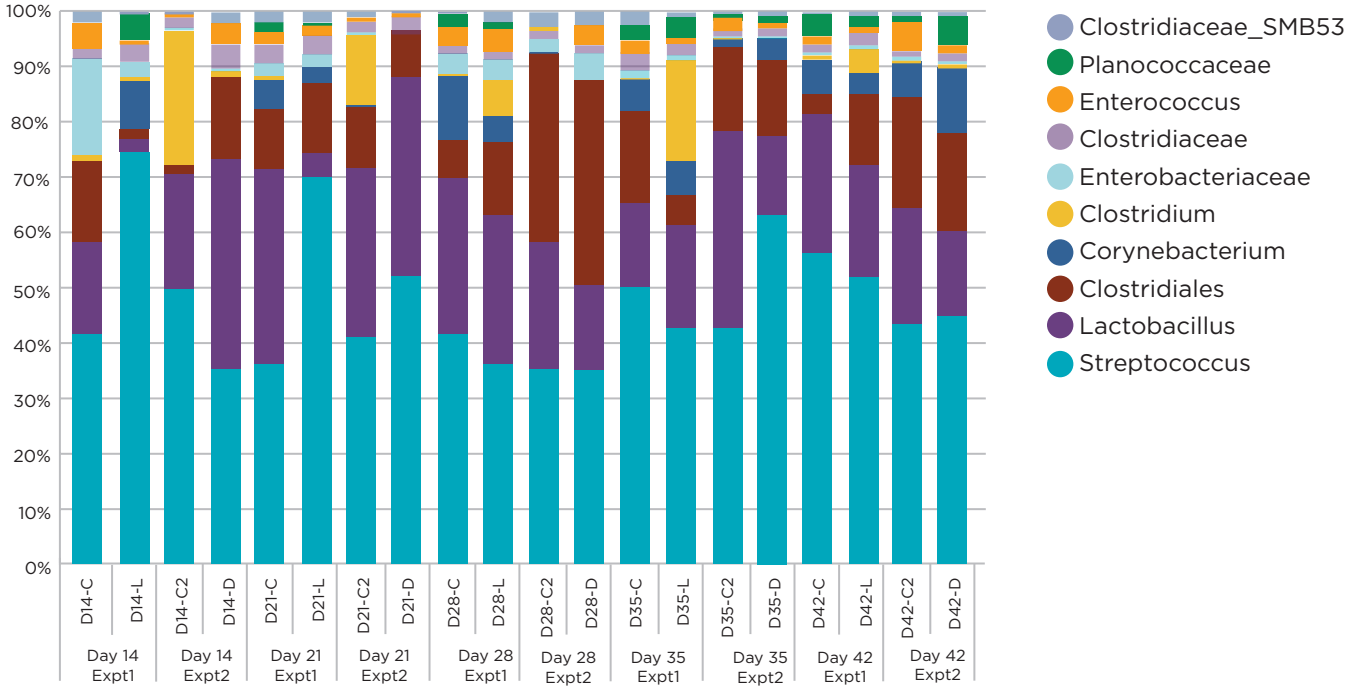
Villi heights and crypt depths were also measured for individual birds from each experimental group at 21 days of age (data not shown). No significant differences in villi height or crypt depth were observed for birds in control versus treatment groups.

The total ileal bacteria for each individual bird in the experiment were assessed using 16S rRNA profiling (figure below). Based upon more than 1.5 million sequences generated (average >5,000 sequences per sample), individual birds, birds according to experimental group, and litter according to experimental group were assessed at each sampling timepoint. Phylum-level analysis of ileum samples revealed that the majority of bacteria at each sampling timepoint in the chicken ilea were comprised of Firmicutes, followed by Actinobacteria and Proteobacteria (Figure 8). There were no significant trends observed comparing ileum bacteria in the control versus treated groups at the phylum level, although differences were often observed between control and treatment groups at each timepoint. At the genus level, differences were also observed between control and Assist-treated groups at certain timepoints, but no overall patterns or shifts in microbiome were observed (Figure 9). Similar results were observed between litter samples of control versus Assist-treated groups; that is, no patterns in microbiome shift between groups were observed while some differences were observed at various timepoints (Figures 10 and 11). Beeswarm plots (Figure 12) were also used at the genus level to determine significant shifts overall between control and Assist-treated groups, independent of timepoint, and the in-water Assist treated groups displayed reductions in *Staphylococcus* and increases in *Coprococcus* and *Brachybacterium*, compared to its respective control group.

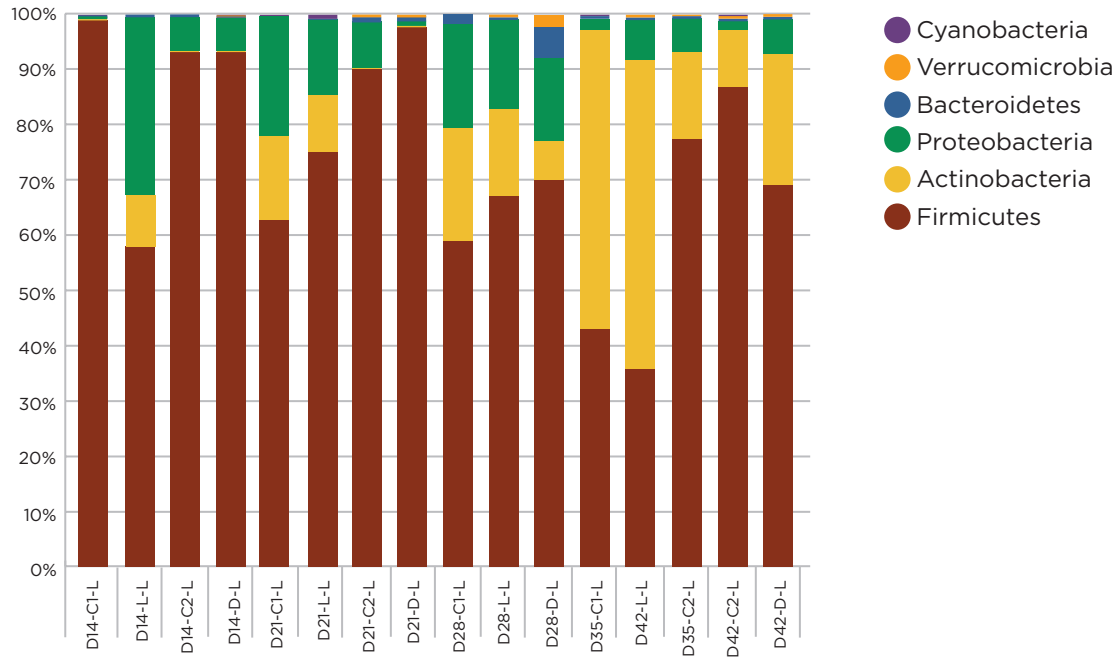
8 Phylum-level bacteria in the chicken ilea of control versus Assist-treated groups at each sampling timepoint.



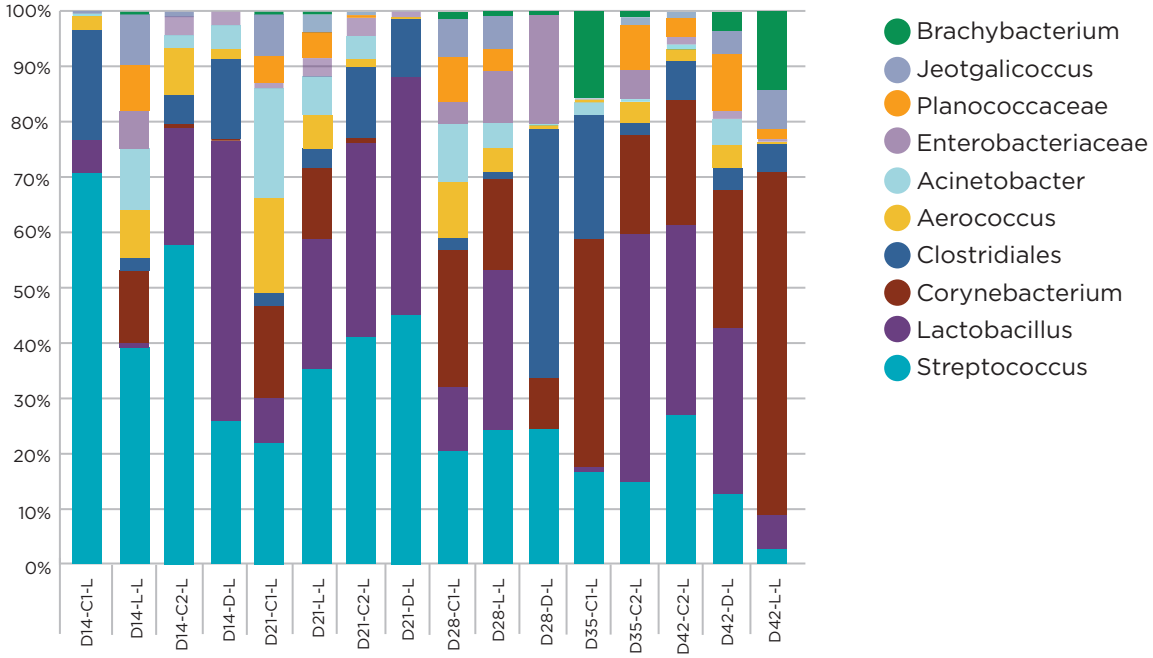
9 Genus-level bacteria in the chicken ilea of control versus Assist-treated groups at each sampling timepoint.

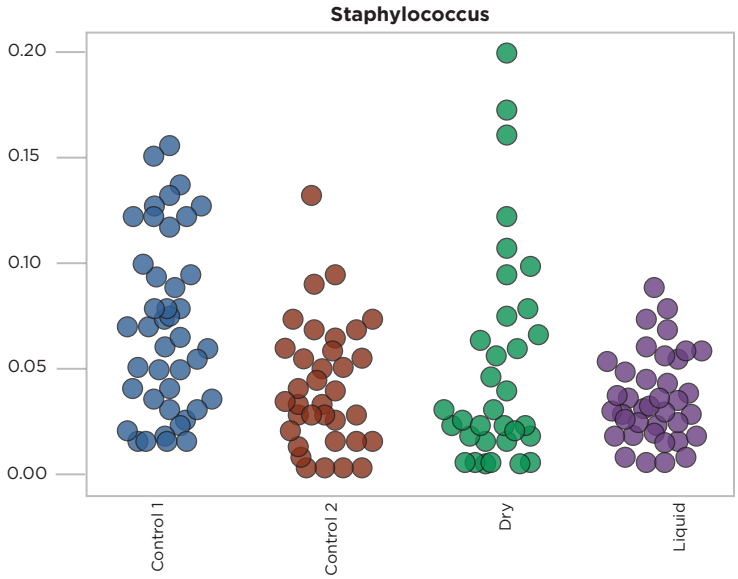


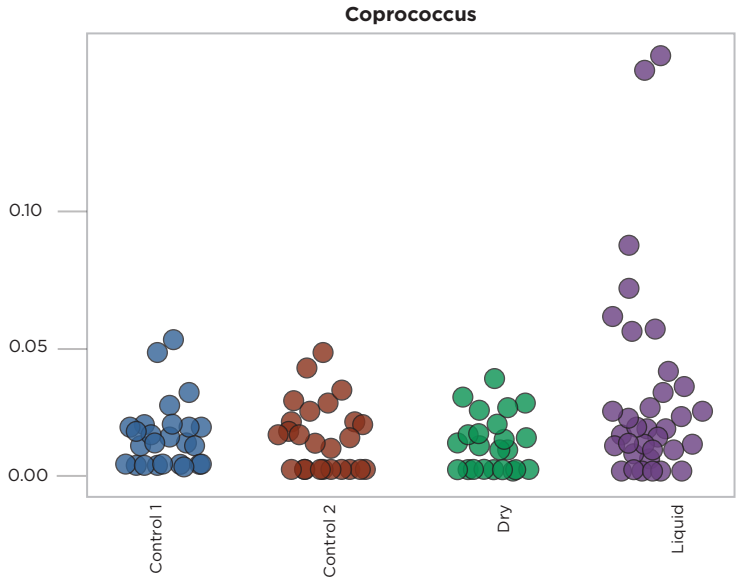
10 Phylum-level bacteria in the litter of control versus Assist-treated groups at each sampling timepoint.

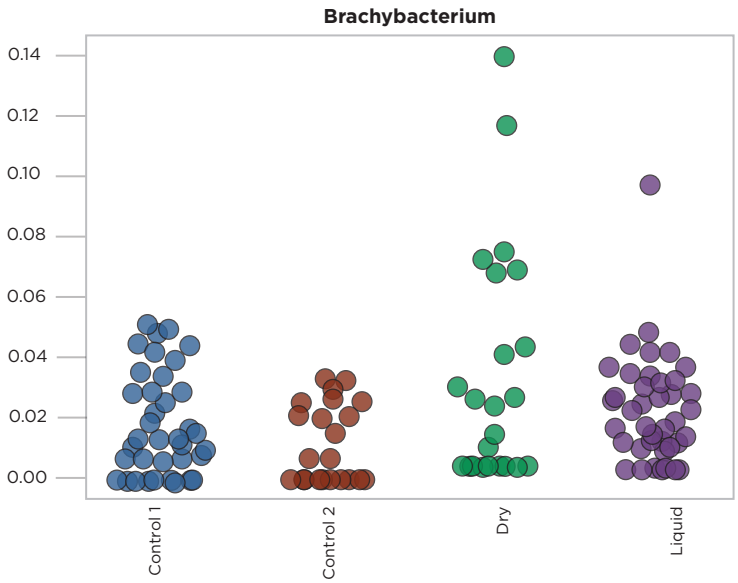


11 Genus-level bacteria in the litter of control versus Assist-treated groups at each sampling timepoint.









12

Beeswarm plots comparing significantly shifted genera independent of time in the control and Assist-treated groups.

CONCLUSIONS

In this study, in-feed and in-water Assist product treatment was tested for its ability to impact bird performance and shift the ileal microbiome over the course of six weeks. The in-feed treatment significantly impacted total bird weights, intestinal weights, and intestinal lengths at later timepoints in the study (35, 42, and 49 days of age), as well as substantial improvement in the FCR over the course of the study. The in-liquid treatment exhibited some, but not all, of these effects, including increased intestinal weights and improvement in FCR. Our measurements of gut development were taken only at 21 days, and the data suggest that significant differences between control and treatment groups did not occur until 35 days of age – therefore, the advantages observed in this study by the Assist program appear to be cumulative over time and are likely amplified over successive flocks. However, the limitations of the study were that successive flocks were not examined, and commercial conditions were not examined to reproduce challenging situations in commercial broiler production. In summary, the in-feed Assist program appears to have substantial potential benefit when applied to commercial broilers related to bird performance and weight gain.

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