College of Veterinary Medicine

University of Minnesota

UNIVERSITY OF MINNESOTA RESEARCH PROJECT:

TESTING THE ASSIST NPS PROGRAM

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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

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 turkey performance and the turkey gastrointestinal microbiota.

MATERIALS AND METHODS

EXPERIMENTAL DESIGN

A total of 148 Hybrid Converter turkey toms 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 (23 birds per pen, 6 pens total). The groups included: 1) poults receiving a standard corn-soy diet; 2) poults 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) poults 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. Procedures regarding the use of the turkeys were approved by the Institutional Animal Care and Use Committee at the University of Minnesota, protocol 1309-30946A.

SAMPLING

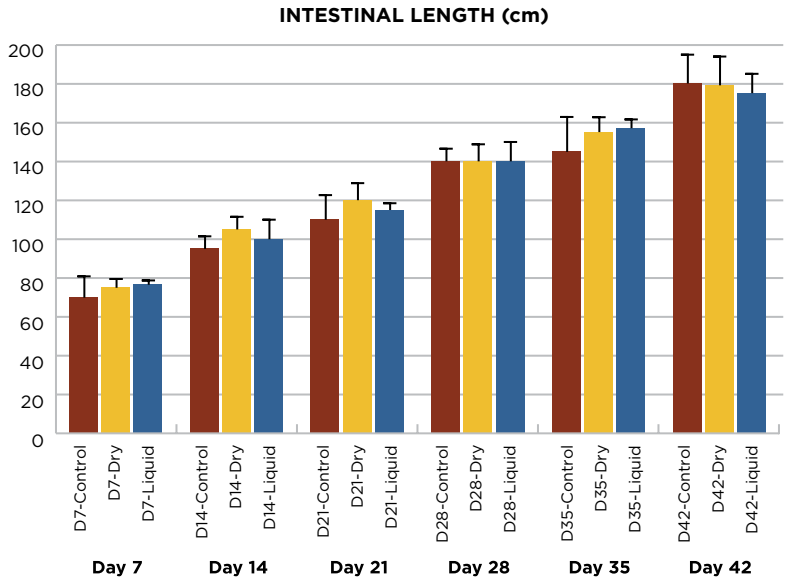
At days 3 and 7 of age, 8 birds per pen were randomly selected and euthanized. Each week following 7 days of age, 6 birds per pen were sampled. Staff were blinded to the experimental groups during bird postings. At euthanization, the following parameters were recorded: total bird weight, total intestinal weight, total intestinal length, and cecal score. 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 $MgCl^2$), 0.2 mM each dNTP (Promega, Madison, WI), 0.4µM 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].

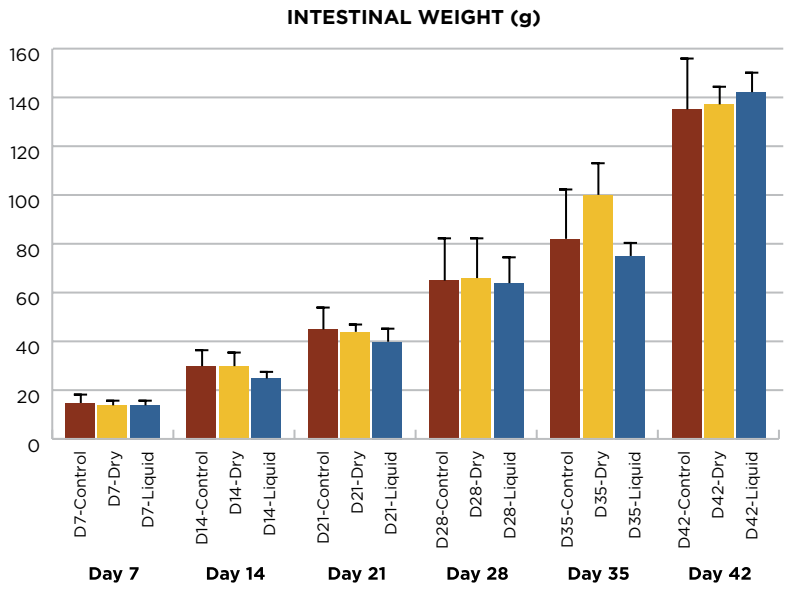


RESULTS

1

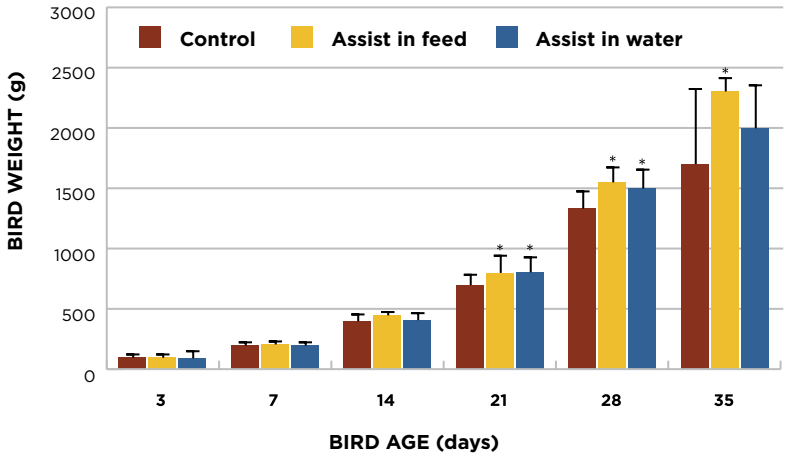
Sampling of intestinal length was performed by measuring length in cm from the proximal end of the small intestine through the ileocecal junction. No significant differences were observed in intestinal lengths at any of the timepoints between control and Assist-treated groups.

Control **Dry** **Liquid**



2

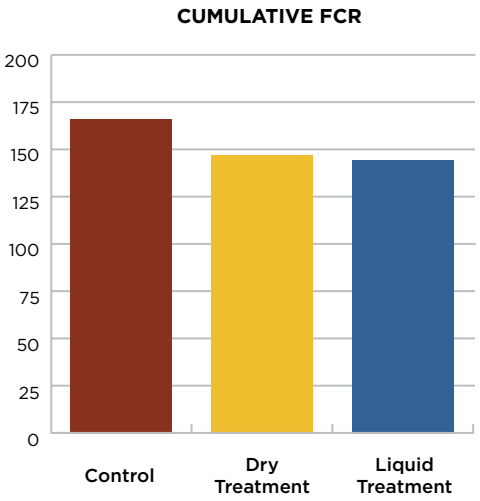
Similarly, intestinal weights were assessed at euthanization by weighing the same section of intestine used for intestinal lengths above. Again, no significant differences were observed in intestinal weights between control versus Assist-treated groups at any timepoint.

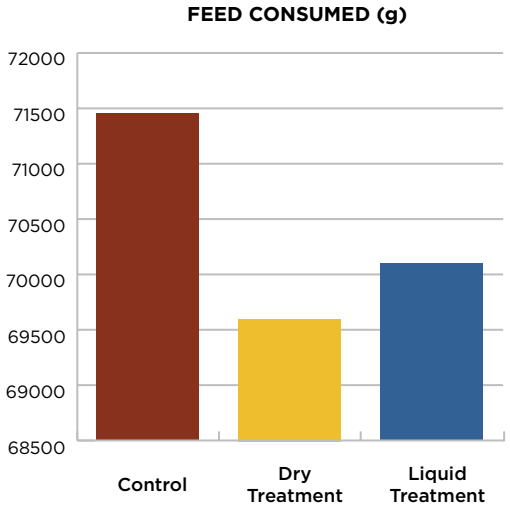


3

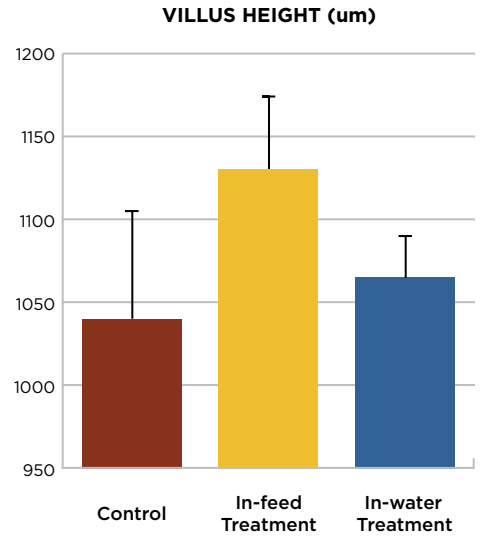
Total bird weights were also measured at euthanization in the control versus Assist-treated groups at each timepoint. At days 21 and 28 of age, both in-feed and in-water Assist treatment groups has significantly higher total body weights ($P<0.05$) than control groups. At day 35 of age, the in-feed treatment group weights were significantly higher ($P<0.05$) than the control group. At all timepoints after day 3, average weights were higher in Assist-treated groups than in the control groups.

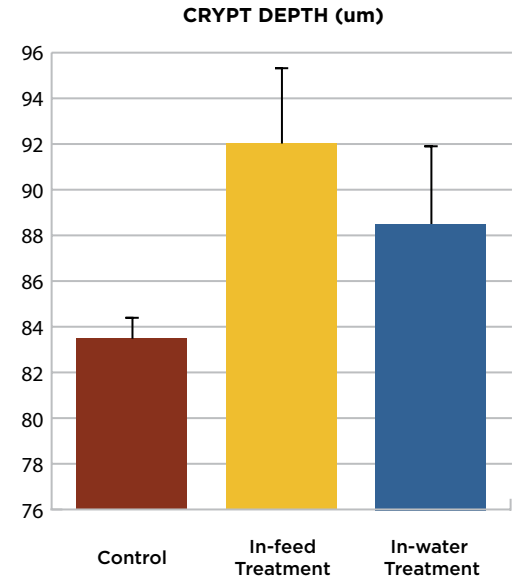
4 Cumulative feed conversion rate (FCR) was assessed by calculating total feed consumed divided by total bird body weights for each experimental group. FCR values were 0.19-0.22 lower in Assist-treated groups compared to the control group.

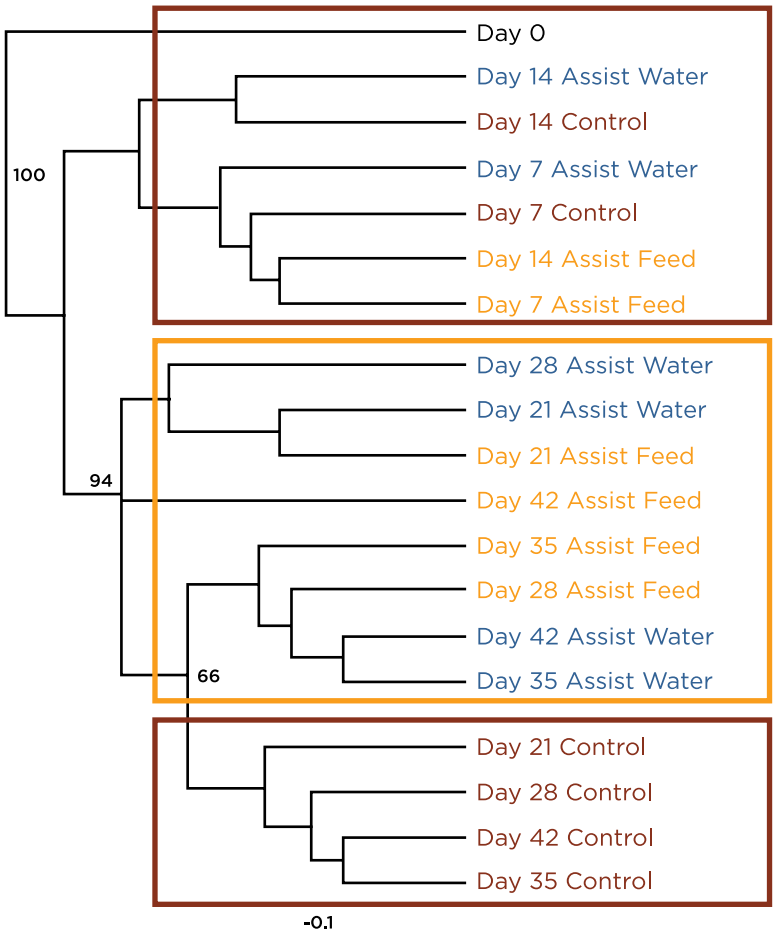




5 Villi heights and crypt depths were also measured for individual birds from each experimental group at 21 days of age. For the in-feed treatment group, both villi heights and crypt depths were significantly larger ($P<0.05$) than for the control group; whereas, the crypt depths for the in-water treated group was significantly larger ($P<0.05$) than the control group at 21 days of age.







6

The total ileal bacteria for each individual bird in the experiment was assessed using 16S rRNA profiling. Based upon more than 1.5 million sequences generated (average >10,000 sequences per sample), a cladogram was generated to depict similarities between the ileal bacterial communities in each group / timepoint. From this analysis, ileal bacterial communities at days 21-42 of age were shifted in the Assist-treated groups compared to control groups of the same timepoints.

WEEK 1

Lactobacillus_aviarius
Lactobacillus_salivarius
Lactobacillus_johnsonii

WEEK 2

Lactobacillus_aviarius
Clostridiales
Lachnospiraceae
Clostridium XIVa

WEEK 3

Lactobacillus_crispatus
Rothia

WEEK 4

Lactobacillus_aviarius
Lactobacillus_johnsonii
Erysilpelotrichaceae
Alistipes
Faecalibacterium
Lachnospiraceae
Rothia
Lactobacillus_oris

WEEK 5

Lactobacillus
Lactobacillus_reuteri
Lactobacillus_reuteri
Lactobacillus_vaginalis
Lactobacillus_reuteri
Lactobacillus_reuteri
Lactobacillus_reuteri

WEEK 6

Lactobacillus_crispatus
Lactobacillus
Clostridium_XI

7

METASTATS was performed to determine members (bacterial species) of each community that were significantly enriched or depleted in the Assist-treated groups at each timepoint in the experiment. At weeks 4, 5, and 6, bacterial species that we have previously associated with enhanced performance in turkeys *(Lactobacillus aviarius, Lactobacillus johnsonii,* and *Clostridium group XI)* were enriched in the Assist-treated samples.

Enriched in the Assist-treated samples

DISCUSSION

Our previous work has demonstrated that specific shifts in the turkey ileal microbiome correlates with enhanced performance in commercial turkeys [11]. Here, the use of Assist Natural Products programmatic approach was applied to commercial turkey toms over the course of 42 days to determine the impact of this approach on bird performance and the gut microbiota. This approach included pre-treatment of litter with an amendment, continuous treatment of water with Pro-Oxine, and continuous administration of a prebiotic and probiotic mixture in feed or water. The results of this work indicate that this treatment significantly improves total bird weights, improves feed efficiency, and positively modulates the ileal bacterial microbiota. While it does not appear that significant colonization of the bacterial species (Bacillus spp.) in the product are colonizing at high rates in the turkey gut, it is evident that this approach modulates the gut, including but gut development and microbiota maturation. It is likely that the effectiveness of these products are through indirect action on the environment and priming of the gut for colonization with beneficial commensal flora.

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