



## College of Veterinary Medicine

UNIVERSITY OF MINNESOTA

UNIVERSITY OF MINNESOTA RESEARCH PROJECT:  
TESTING THE ASSIST NPS PROGRAM

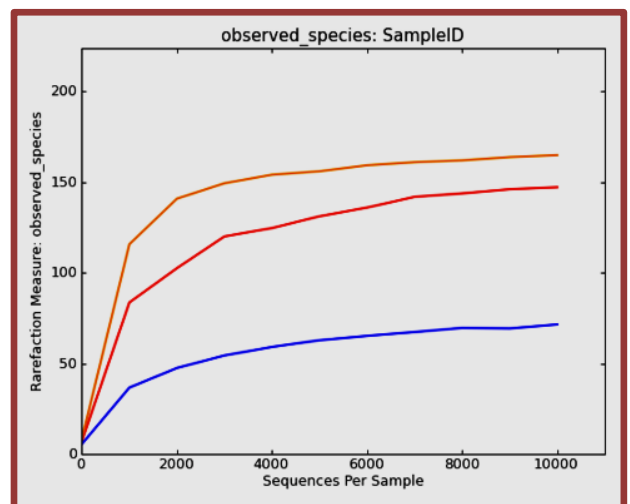
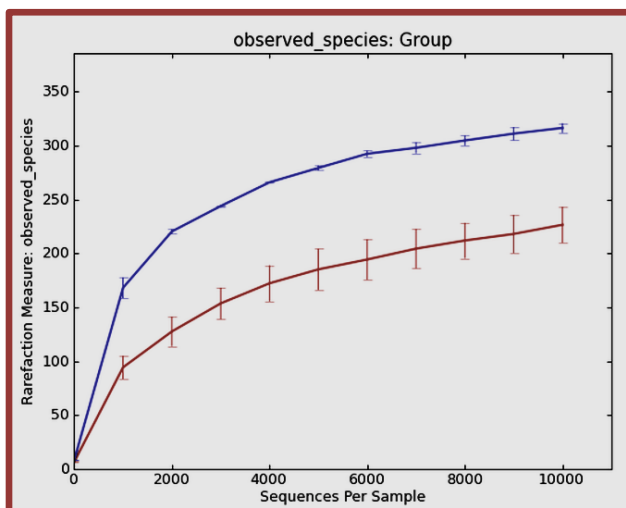
**COMBINED REPORTS FROM JULY 13 AND DECEMBER 1, 2015**

### Report for Assist Natural Products, combined experiments for testing manure from turkey flocks

A total of 7 samples were tested, composed of pooled litter/fecal samples from control (n=3), treatment (n=3), and conventional (n=1) turkey flocks. DNA was processed from these samples using a standard bacterial DNA isolation kit. PCR was performed on each sample for the V4 region of the 16S rRNA gene for bacterial community profiling. Sequencing was performed on each sample to depths of approximately 100,000 reads per sample. Sequencing reads were quality filtered and analyzed in the QIIME software.

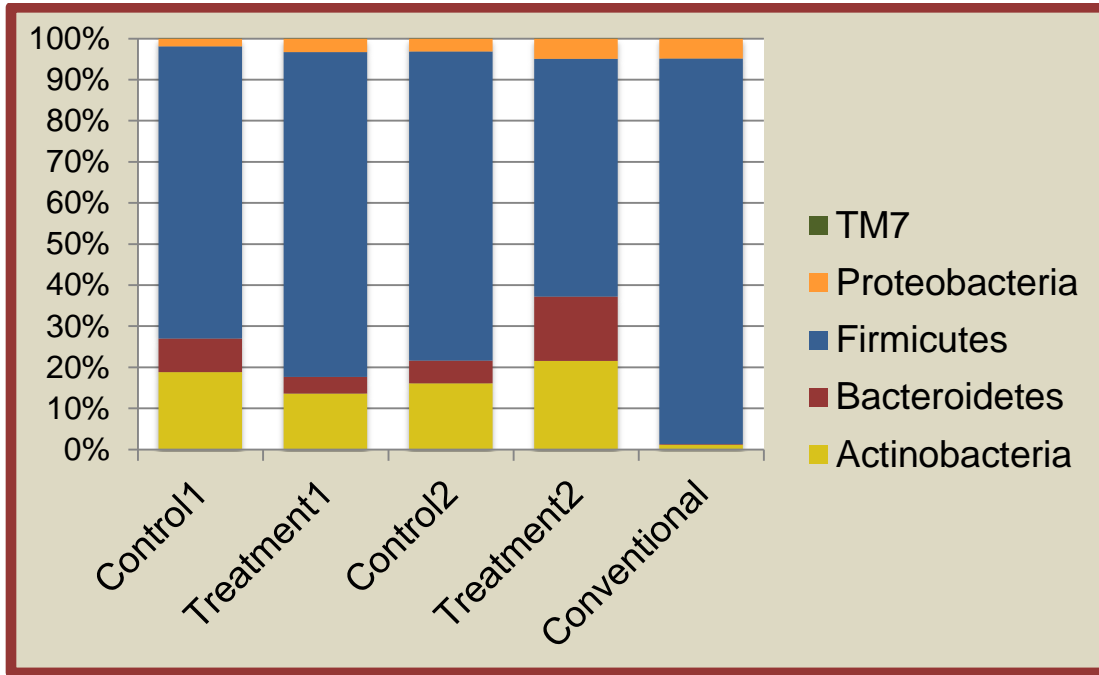
#### Bacterial community diversity:

The below plot depicts alpha diversity of the bacterial communities from the samples analyzed. On the left chart is experiment #1, control (red) versus treatment (blue) samples. On the right chart is experiment #2, conventional (blue) versus control (red) versus treatment (orange). These plots depict sequences sampled on the x-axis and number of new species observed on the y-axis. More diverse bacterial communities, which are considered a hallmark of a healthy gut microbiome, will appear higher on the chart than less diverse communities. The plot shows that the bacterial communities in the treatment samples were significantly more diverse than those of the control samples ( $P < 0.001$ , left and right) and of the conventional sample (right).

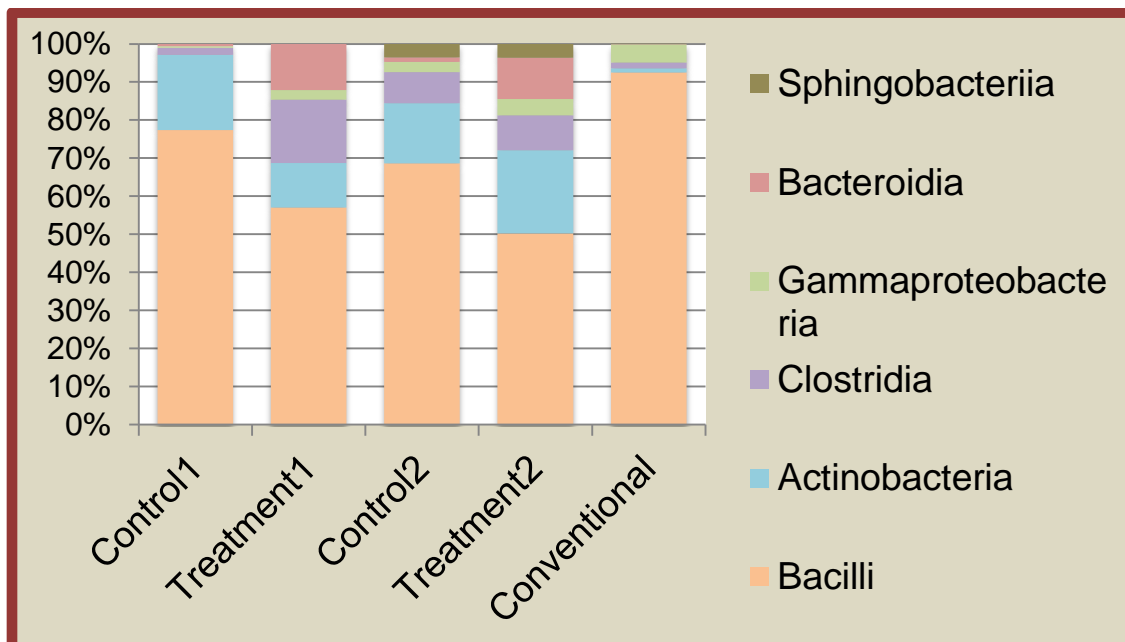


### Bacterial community composition:

The specific bacterial taxa differentiating control, treatment, and conventional groups were also examined at the phylum and class levels (the higher levels of taxonomic classification). The table below lists bacterial phylum-level differences between control and treatment groups. In both experiments, differences exist between control versus treatment samples, and all samples are distinct from the conventional sample.



The next chart shows differences in taxonomic classification at the bacterial class level. In this chart, it is evident that the treatment groups have bacterial communities that are more diverse, comprised of more bacterial classes that are enriched in the treatment samples. This illustrates that treatment flocks have higher proportions and greater numbers of healthy bacterial species in the flock environment.



## Bacterial species-level analysis:

When broken down by bacterial species, a number of species were identified that differ between control and treatment and/or conventional groups. Red-colored numbers are bacteria reduced in the treatment group relative to its control, and blue-colored numbers are bacteria increased in the treatment group relative to its control. Shown are the top 15 dominant bacterial species identified across samples. A number of bacterial species that have been associated with enhanced gut health were increased in the treatment group, including *Lactobacillus* spp., *Prevotella* spp. and *Clostridium bartletti*. The latter is a known marker of turkey gut maturation that appears earlier in healthier flocks. Species highlighted in green are those where trends were observed in both experiments.

Bacteria	Control1	Treatment1	Control2	Treatment2	Conventional
<i>Lactobacillus crispatus</i>	8209	7056	3338	5635	7509
<i>Lactobacillus aviarius</i>	6511	5767	7044	1666	6633
<i>Corynebacterium</i>	3918	2707	2439	3866	54
<i>Streptococcus</i>	1125	3047	5073	597	281
<i>Lactobacillus johnsonii</i>	1411	1672	165	638	4297
<i>Brachyбактерium</i>	1236	900	1556	2904	75
<i>Staphylococcus</i>	661	871	313	448	7
<i>Lactobacillus salivarius</i>	711	457	210	915	2247
<i>Jeotgalicoccus</i>	662	312	143	449	20
<i>Enterococcus</i>	531	487	3859	5161	18
<i>Jeotgalicoccus</i>	731	182	140	980	2183
<i>Prevotella</i>	0	1014	9	928	0
<i>Lactobacillus reuteri</i>	334	505	208	313	1489
<i>Escherichia coli</i>	55	745	252	127	1301
<i>Clostridium bartlettii</i>	127	500	1475	1595	109

## Conclusions:

The common observation in this study is that treatment samples in both experiments had more diverse bacterial communities than control group samples, suggesting a healthier and better functioning microbiome in the bird and its surrounding environment. The treatment samples also differed in their overall bacterial species content and contained increased numbers of marker species of a healthy turkey gut.

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