# EFFECT OF LACTOBACILLUS SALIVARIUS L28 USED AS A FEED ADDITIVE ON THE GUT MICROBIOME OF FEEDLOT CATTLE

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## I. INTRODUCTION

The microbial community present in the animal's gastrointestinal tract is critical in maintaining the animal's health and productivity [1]. Rumen microbiota is critical for proper digestion of feeds to meet energy requirements [2]. Antimicrobials such as tylosin, monensin and direct-fed microbials (DFM) are feed additives that have been widely used in livestock production systems as growth promoters to improve animal performance, daily gain, while also reducing the shedding of pathogenic bacteria such as *Escherichia coli* O157:H7 [3,4]. However, more research is needed to analyze the effect of a diet utilizing a DFM or sub-therapeutic antibiotics on the shift of the microbial community [2]. Thus, the objective of this study was to assess the effect of feeding a DFM-supplemented diet, with and without antimicrobials, on the shift in the microbial community, composition and abundance over time in feedlot cattle through microbiome analysis.

## II. MATERIALS AND METHODS

A total of three dietary treatments based on conventional high concentrate diets were fed to finish cattle for harvest: CONTROL containing tylosin (88 mg/hd/d of diet dry matter (DM)), and monensin (330 mg/hd/d of diet DM); MONPRO containing a newly isolated DFM, *L. salivarius* L28, at a feeding rate of $10^6$ CFU/hd/d, monensin (330 mg/hd/d of diet DM), and no tylosin; and BASE which had no DFM, tylosin or monensin. For microbiome analysis 100 g of fecal was collected rectally, from 12 animals per treatment on days 0 and 140. Samples were stored at -80°C until further analysis. DNA was extracted from fecal samples using the QIAamp DNA Mini Stool Kit following manufacturer's recommendations. Pure DNA from each sample was used to prepare libraries using the Illumina 16S-metagenomics library preparation protocol by the PCR amplification of the V3-V4 region of the 16S rRNA genes. An indexed library was prepared for each sample using the Nextera XT index kit v2, PCR amplicons were then purified using AMPure XP beads. Cleanup products were quantified in triplicate using a Qubit 2.0 fluorometer, pooled at equal concentrations, and finally diluted to a final concentration of 4.5 pM. Samples were sequenced using a MiSeq Reagent kit v3 (600 cycle) on an Illumina MiSeq sequencer. Significance was determined using the Wilcoxon signed-rank test and the DeSeq package in R with $p < 0.05$ determined as significant.

## III. RESULTS AND DISCUSSION

Relative abundance of bacteria in the phyla *Bacteroidetes* increase over time with the overall reduction in relative abundance of all other bacteria. There were no changes based on treatment following application of either control or MonPro regiments see Figure. 1. For the base treatment group there was the greatest changes over time. Figure 2 shows the statistically significant ($p < 0.05$) differences for the MonPro treatment compared to the control on 0 and 140 d of treatment and the bacterial taxa abundance.

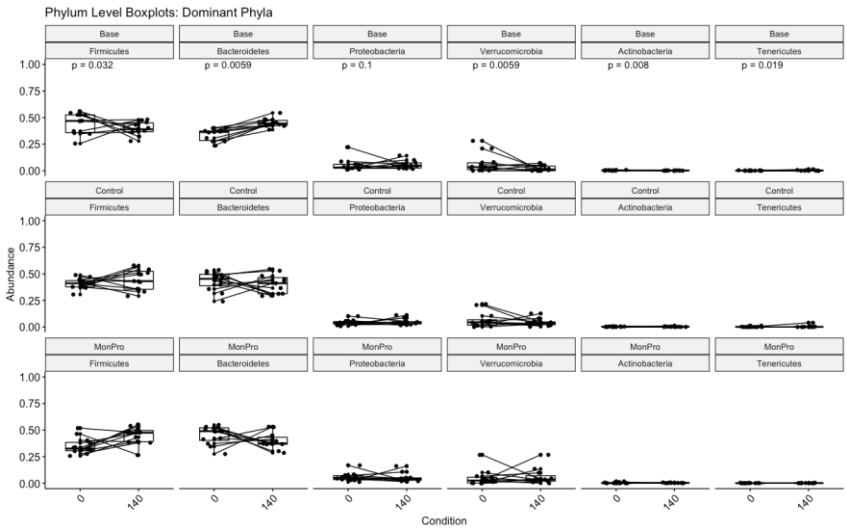


Figure 1. Comparison of the relative abundance of bacterial phyla pre- and post-treatment.

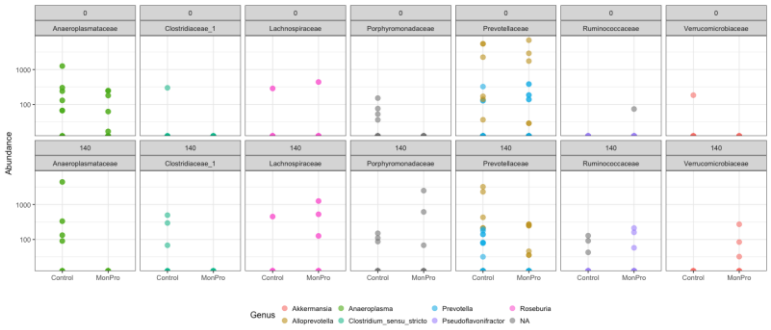


Figure 2. Bacterial taxa differentially abundant between control and MonPro.

## IV. CONCLUSION

*Bacteroidetes* and *Firmicutes* are two of the most dominate phyla in the gut of microbial community of most mammals, which corresponds with our results that *Bacteroidetes* were in the greatest abundance in gut microbiota regardless of treatment [5]. There was also no diet effect on the overall abundance in the microbial community.

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