

Abstract

Feedlot cattle that receive high grain diets are prone to developing rumen acidosis. The rumen microbiome is a critical part of feed digestion, and thus naturally occurring, native rumen microorganisms may alleviate digestive distress. A daily, in feed microbial feed supplement (MFS) (Magnius, ASCUS Biosciences Inc, San Diego, California) containing three native rumen microbes (*Chordacoccus ruminofurens*, *Prevotella albensis*, and *Succinivibrio dextrinosolvens*) was evaluated. Seventy-five commercial feedlot steers (Johnson Research, LLC, Parma, Idaho) were split into three treatment groups, 25 control, 25 low-dose (3E8 cells/steer/day), and 25 high-dose (3E9 cells/steer/day). The study included three periods: acclimation (28 days), grow-out period 1 (68 days), and grow-out period 2 (73 days). The animals were individually penned for the acclimation and grow-out period 1 and were collapsed into 7-9 steers per pen for grow-out period 2. The finishing ration for grow-out period 2 was also adjusted to 89.77% concentrate dry matter from 85.56% during grow-out period 1.

No significant performance differences among treatment groups were observed during grow-out period 1. In grow-out period 2, the average daily weight gain of mid-weight steers was significantly higher ($p = 0.04$) in animals receiving a high-dose of MFS than controls. Additionally, the rumen pH of experimental steers were significantly higher than the controls ($p < 0.001$) during grow-out period 2. The rumen microbiome shifts further supported the observed pH differences. A strong ($R^2 > 0.6$) and significant negative correlation ($p < 0.001$) was observed between rumen pH and rumen dissolved %CO₂ in all three treatment groups throughout the study. This is consistent with literature reporting that rumen CO₂ accumulation may contribute significantly to rumen acidosis. These findings demonstrate the promise of using microbial based feed supplements in the improvement of feedlot cattle digestive health and performance.

Experimental Design

Figure 1. Experimental Period Timeline

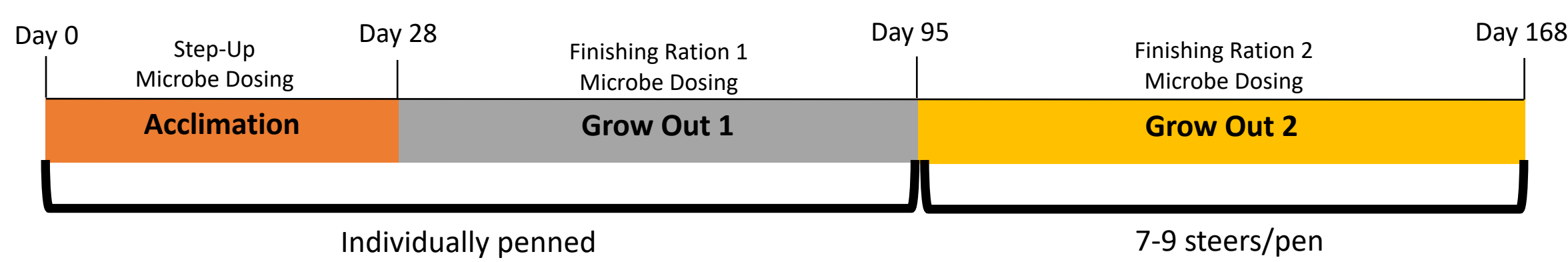
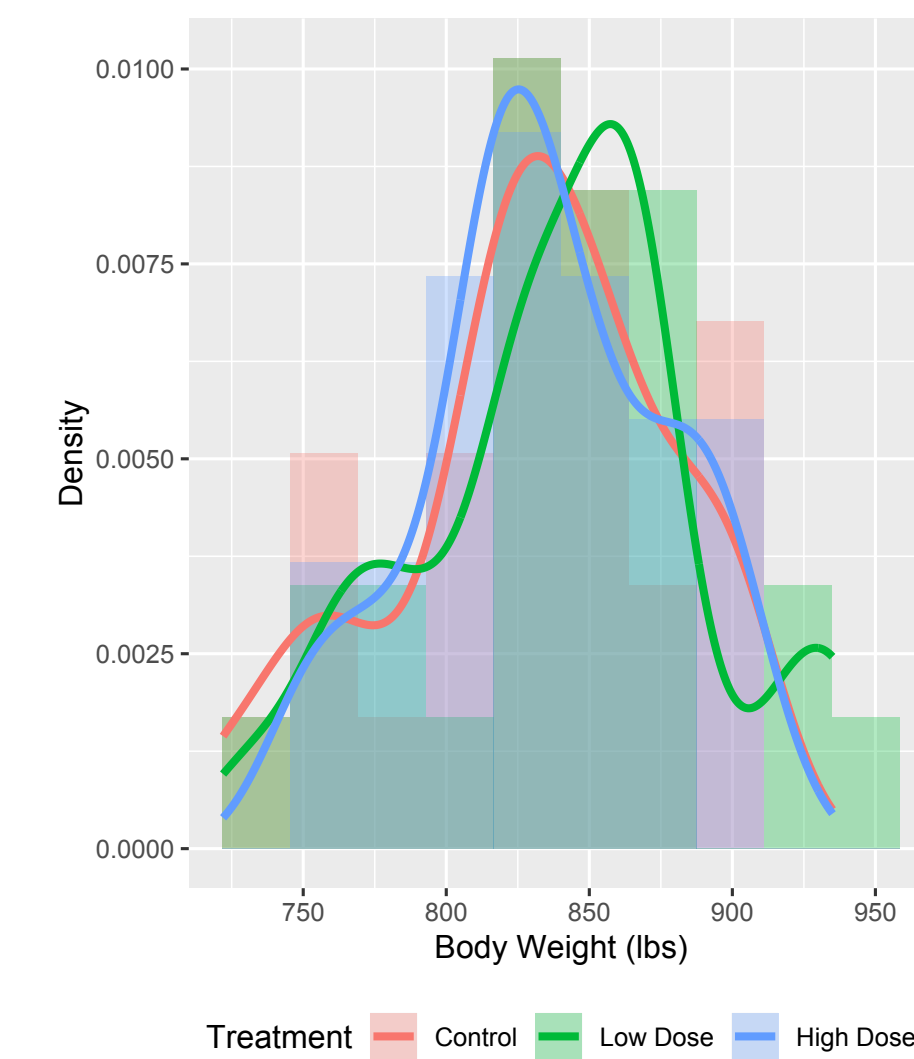


Figure 2. Initial Body Weight Distribution



- ① 75 steers:
 - ① 25 control (CaCO₃ carrier without microbes),
 - ② 25 low-dose (3E8 cells/steer/day),
 - ③ 25 high-dose (3E9 cells/steer/day)
- ② Product mixed into feed daily, animals fed ad-libitum
- ③ 1-week covariate prior to Day 0
- ④ Finishing Ration 2 more fermentable than Finishing Ration 1

Measurements

Physiological measurements:
Body Weight (~monthly)
Weigh backs / FCR

Rumen measurements:

pH
dCO₂
VFAs (in progress)
Microbiome composition

Table 1. Feed Composition Across All Stages

Item		Grass					
		Hay ration	Step 1 ration	Step 2 ration	Step 3 ration	Finish ration 1	Finish ration 2
Ration Starting Day		0	14	22	28	95	
Ration Dry Matter	%	74.6	69.66	71.79	70.66	69.41	72.37
Fat	%	2.65	3.33	4.86	5.11	6.45	6.29
ADF	%	32.97	17.91	16.93	14.73	12.07	11.35
NDF	%	54.04	27.9	28.47	26.54	23.84	22.02
Crude Protein	%	16.13	14.51	13.84	13.25	13.35	12.86
NEm	Mcal/cwt	55.39	79.1	84.42	88.92	95.39	95.65
NEg	Mcal/cwt	29.71	51.1	56.64	60.7	67.23	67.23
Monensin	g/ton	20.17	25.93	31.24	32.38	39.9	0
Tylosin	g/ton	4.09	5.25	6.33	6.56	8.09	0
Forage Dry Matter	%	91.42	44.63	34.33	24.46	14.44	10.23
Concentrate Dry Matter	%	8.58	55.37	65.67	75.54	85.56	89.77

Performance Results

Figure 3. Rumen CO₂ Influences Rumen pH

Dissolved carbon dioxide (dCO₂) is a major by-product of microbial feed fermentation in the rumen and can be converted to a strong acid, carbonic acid (H₂CO₃). The speciation of dCO₂ is governed by physicochemical properties of rumen and influences pH. Uribe (2016)¹ reviewed that rumen pH can be explained by the equilibrium of dCO₂ and HCO₃⁻, where a lower pH could lead to the dominance of dCO₂ and vice versa. Therefore, decreasing in rumen pH due to rapid fermentation could lead to a greater amount of dCO₂ in rumen and further exacerbate the decrease in pH.

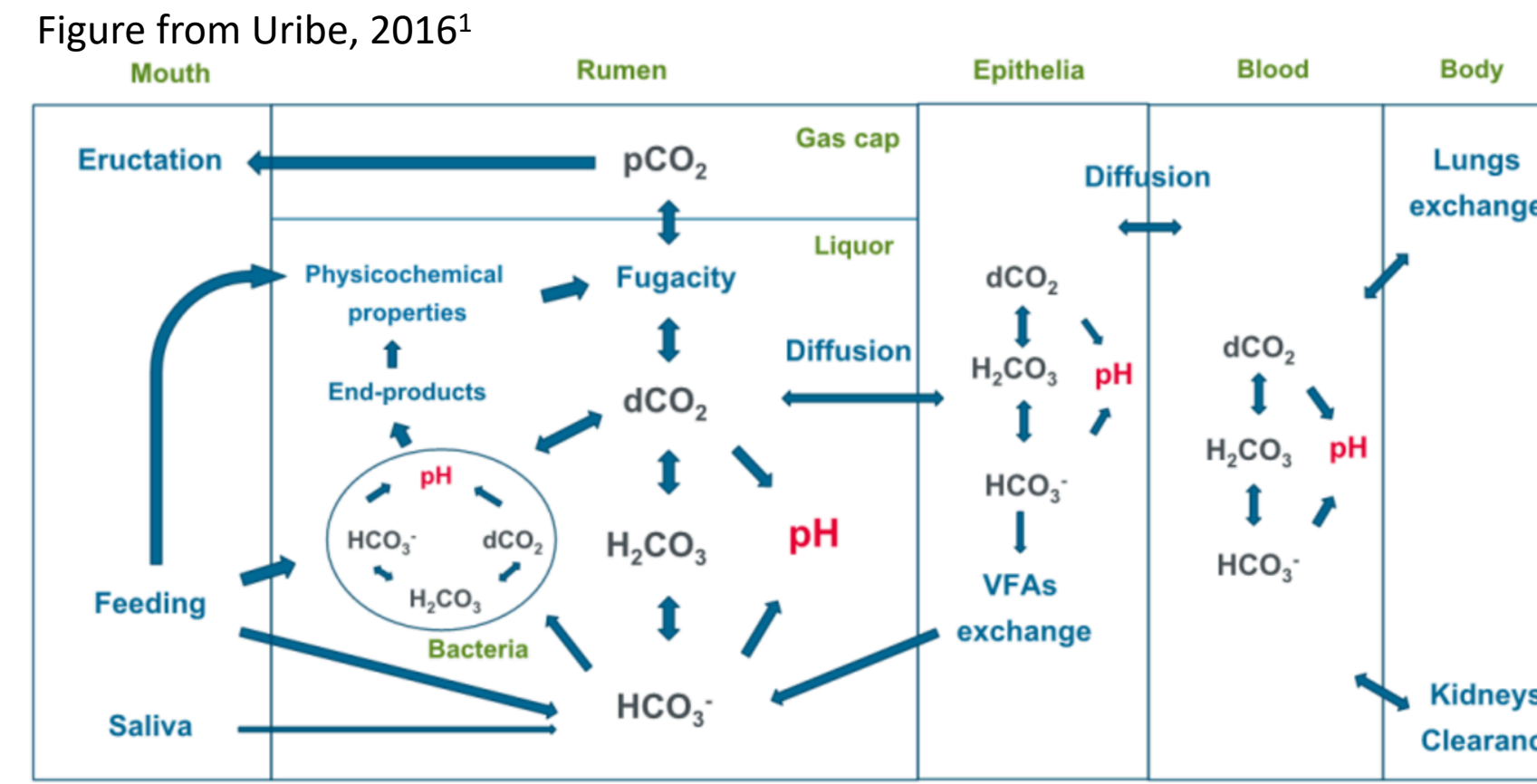


Figure 4. Rumen pH Throughout the Trial

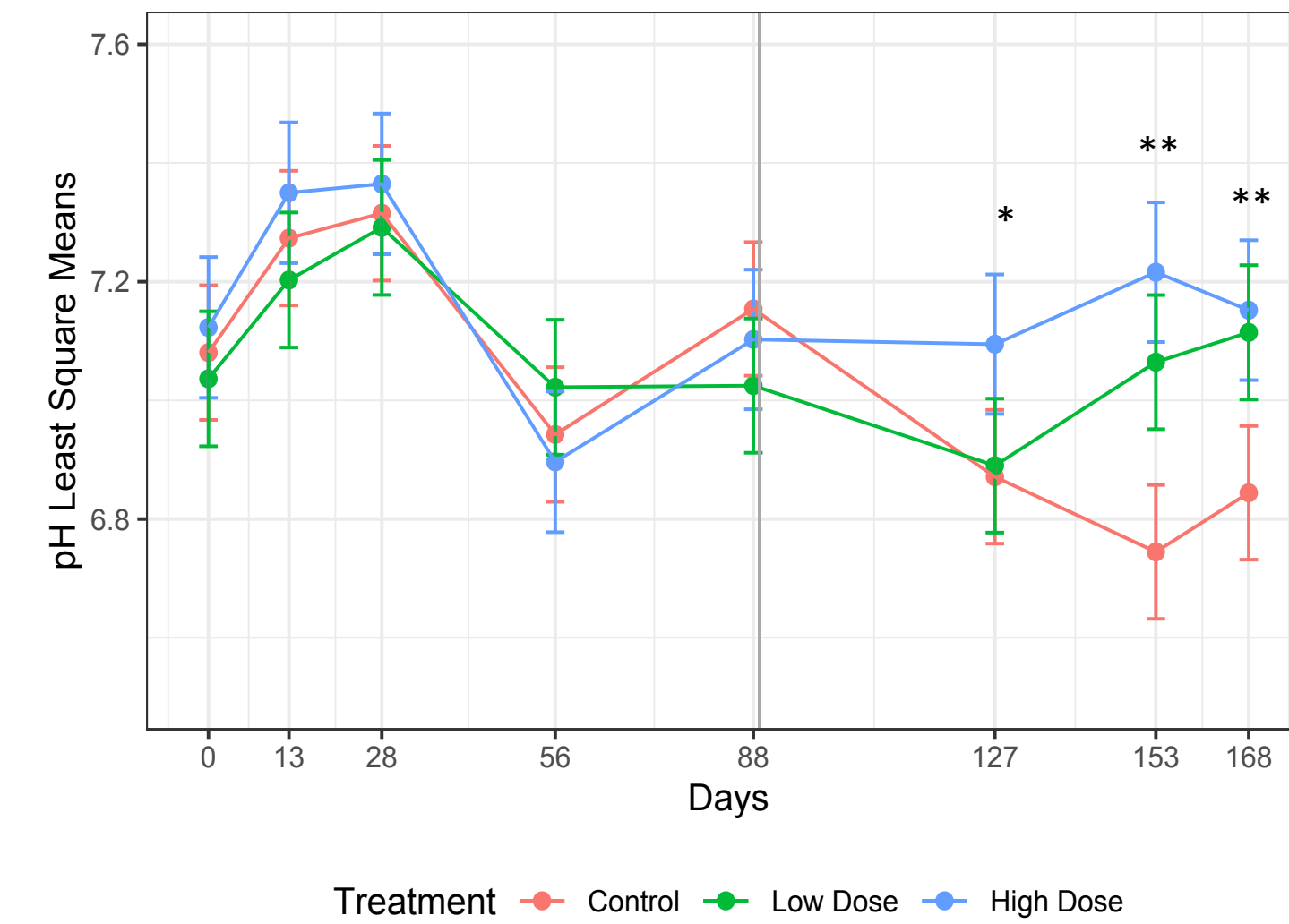


Table 2. Treatment p-values at Each Sampling Point

p-values		Control - Low Dose	Control-High Dose	Low Dose-High Dose
		Control	Low Dose	High Dose
Acclimation	day 0	0.85	0.86	0.55
	day 13	0.66	0.62	0.18
	day 28	0.95	0.82	0.65
Grow Out 1	day 56	0.59	0.84	0.29
	day 88	0.25	0.80	0.61
	day 127	0.97	0.02	0.04
Grow Out 2	day 153	< .001	< .001	0.16
	day 168	< .001	< .001	0.89

Figure 6. Animal Average Daily Weight Gain and FCR for Each Experiment Period

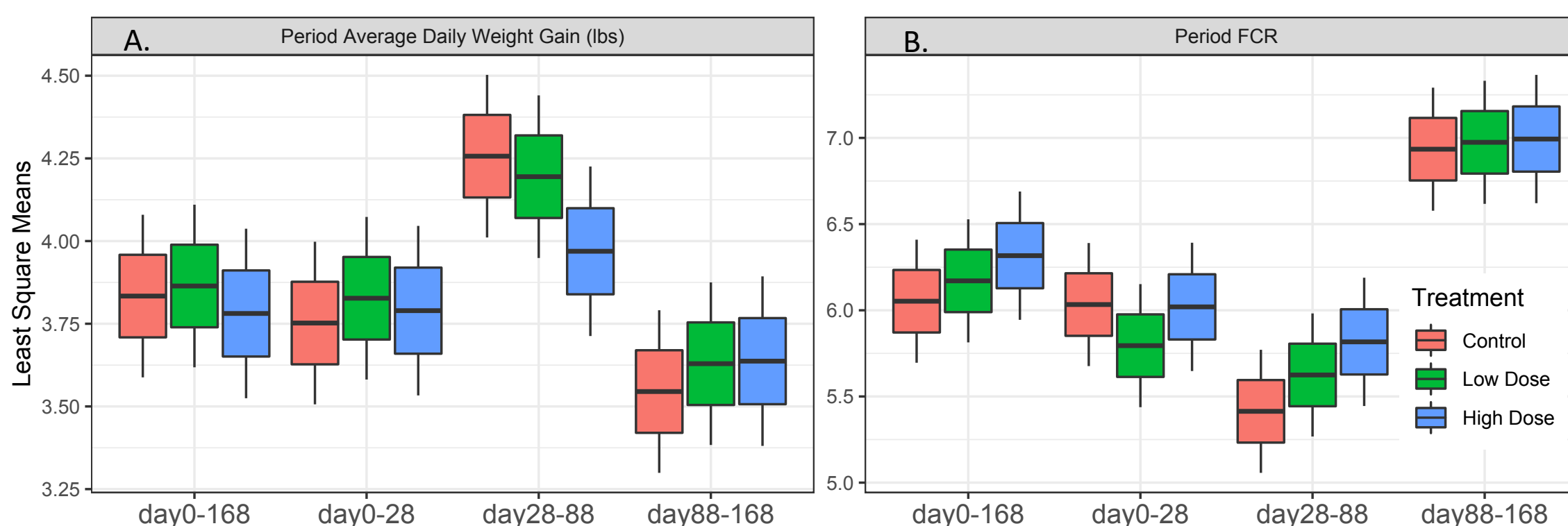


Table 3. Treatment p-values for Each Experiment Period

p-values		Average Daily Weight Gain (lbs)			FCR		
		Control - Low Dose	Control-High Dose	Low Dose-High Dose	Control - Low Dose	Control-High Dose	Low Dose-High Dose
Whole Trial	day 0-168	0.98	0.95	0.89	0.89	0.57	0.84
	Acclimation	day 0-28	0.91	0.98	0.98	0.62	1.00
Grow Out 1	day 28-88	0.93	0.25	0.42	0.69	0.27	0.74
	Grow Out 2	day 88-168	0.88	0.87	1.00	0.99	0.97

1. Laporte Uribe, José. (2016). The role of dissolved carbon dioxide in both the decline in rumen pH and nutritional diseases in ruminants. Animal Feed Science and Technology. 219. 10.1016/j.anifeeds.2016.06.026.

Microbiome Analysis

Figure 7. Alpha Diversity (Species Diversity at Each Sampling Point)

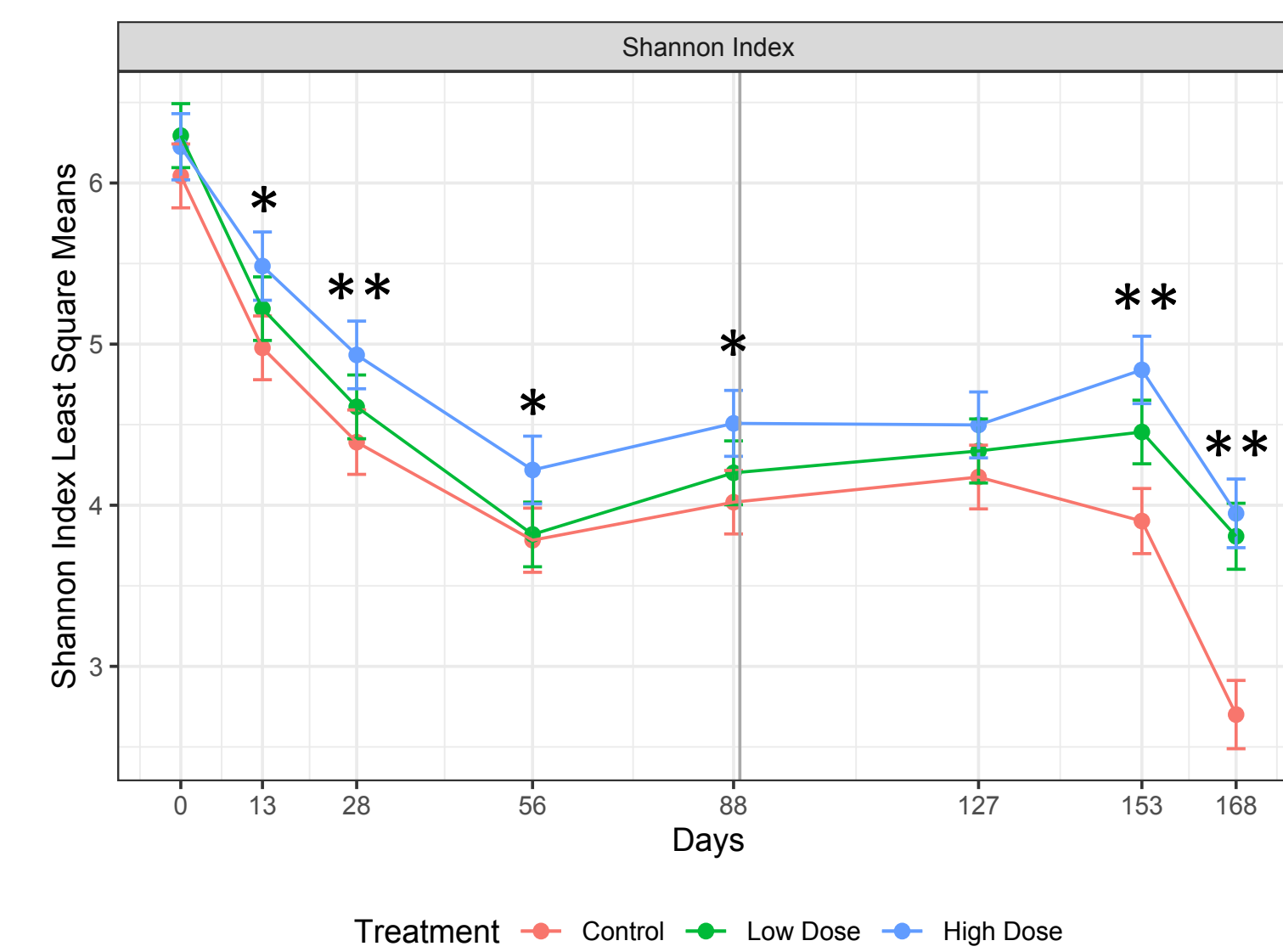


Table 4. Treatment p-values for Each Sampling Point

p-values		Control - Low Dose	Control-High Dose	Low Dose-High Dose
		Control	Low Dose	High Dose
Acclimation	day 0	0.18	0.42	0.88
	day 13	0.20	0.003	0.17
	day 28	0.27	< .001	0.07
Grow Out 1	day 56	0.97	0.01	0.02
	day 88	0.40	0.003	0.09
	day 127	0.48	0.07	0.50
Grow Out 2	day 153	0.001	< .001	0.03
	day 168	< .001	< .001	0.58

Figure 7 (left) shows the changes in rumen microbial alpha diversity, expressed as Shannon index during the trial. Rumen samples from animals administered microbes had a greater Shannon index than control samples, suggesting the microbial treatment increased both rumen microbial diversity and evenness. The symbol "*" denotes a significant p-value < 0.05 and "***" denotes a significant p-value < 0.001 between control and microbial treated samples. Table 4 (above) shows all treatment p-values. The significant p-values are bolded and italicized.

Figure 8. The Shifts of Dominant Bacteria (Order Level)

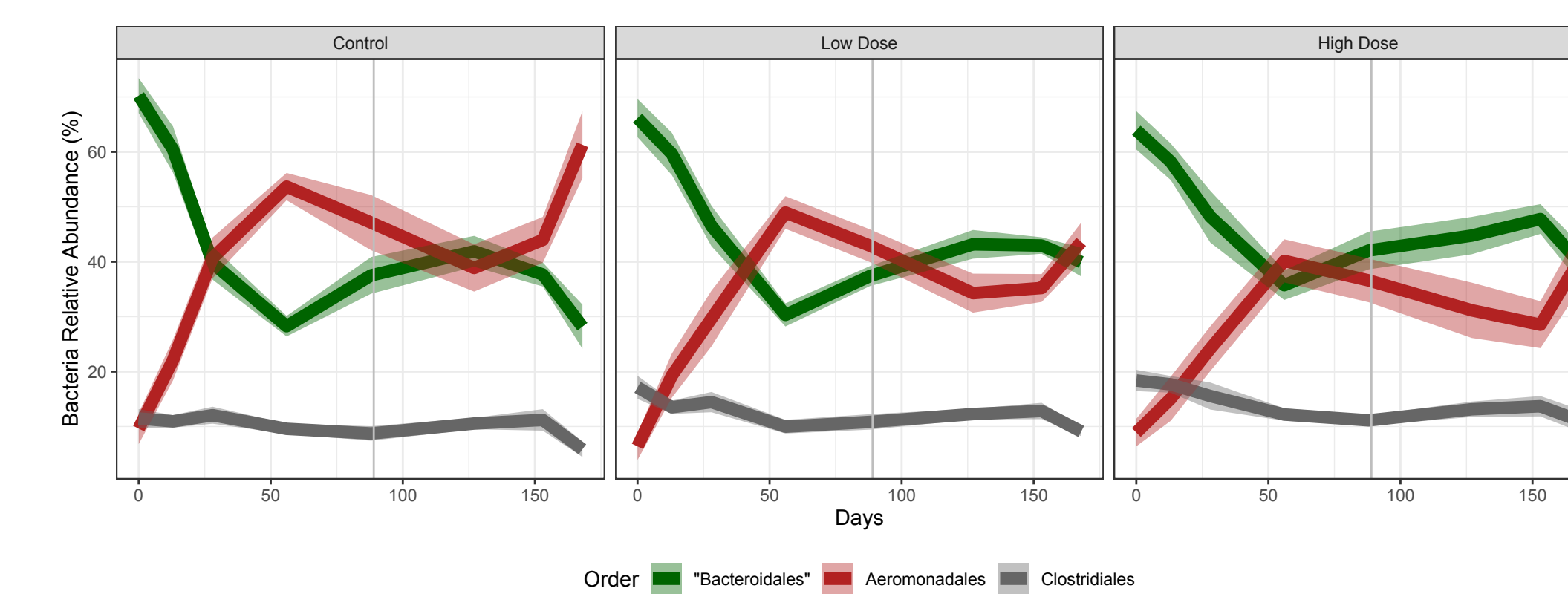


Figure 8 (left) shows the relative abundance of the top 3 most dominant bacteria orders in control, low dose, and high dose samples throughout the trial. Rumen samples from animals that received microbes maintained a stable Bacteroidales-Aeromonadales relationship compared to the control animals. Members of these top three bacteria orders have been linked to feed conversion and dCO₂ consumption.

Figure 9. Beta Diversity (Difference in Diversity Between Samples)

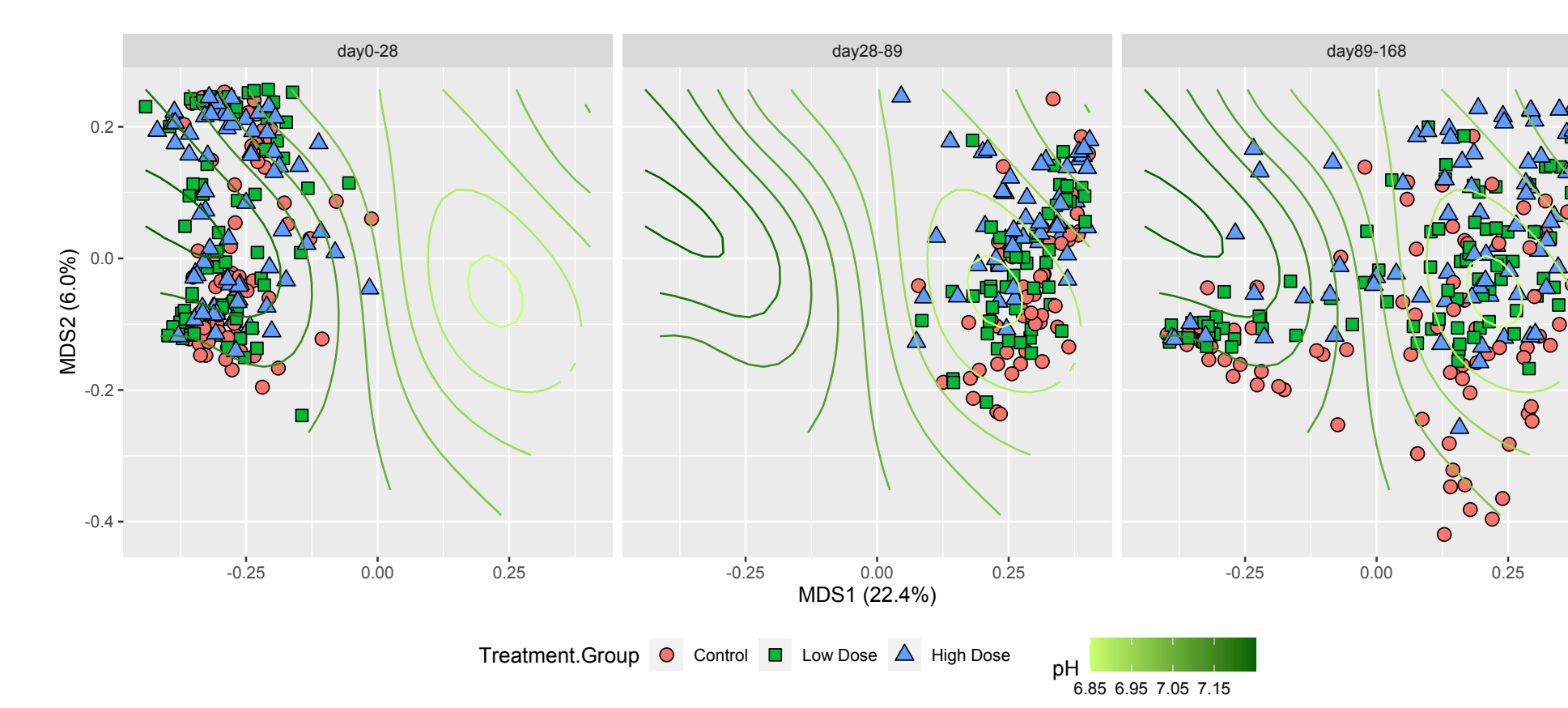


Figure 9 (left) shows the bacterial order composition differences during acclimation (day 0-28), grow out 1 (day 28-89), and grow out 2 (day 89-168) using multidimensional scaling (MDS). Each point represents a rumen sample. The dissimilarities among samples were calculated using Bray-Curtis distance and displayed in two dimensions (MDS1 and MDS2). A greater separation of points represent a greater microbiome differences. The cumulative percent variances on the axes show the amount of community differences captured by the two dimensions. The gradient lines are fitted with rumen pH to show the correlation between rumen pH and microbiome composition.

Conclusions

- ① No significant performance differences were observed between treated and control steers. However, the least square means of average daily weight gain were greater in the treatment groups than the control during Grow Out 2. Rumen pH was significantly higher in all steers receiving microbes during Grow Out 2.
- ② Rumen pH negatively correlated with rumen CO₂ throughout the experiment, showing that CO₂ may influence pH as much (if not more) than VFA and lactic acid concentrations.
- ③ The temporal shifts among Bacteroidales and Aeromonadales support observations from previous experiments that rumen CO₂ levels are increasing as animals consume higher grain diets.
- ④ The rumen microbiome became more skewed throughout the experiment, but treated samples were able to regain their evenness and maintain a higher rumen pH.

○ VFA analysis and methanogen analysis still underway.