

FEED EFFICIENCY AND THE MICROBIOTA OF THE ALIMENTARY TRACT
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The casual mechanisms for variation in feed efficiency are poorly described

- Digestion
- Metabolism

Why gut microbes

- Gut microbial communities have been demonstrated to change in obese animals
- The gut microbes have the opportunity to use nutrients in feed before the animal
- Dietary energy is lost to the environment through fermentation

Hypothesis

Cattle that differ in feed efficiency have differences in the microbiota of the alimentary tract

Two studies were conducted

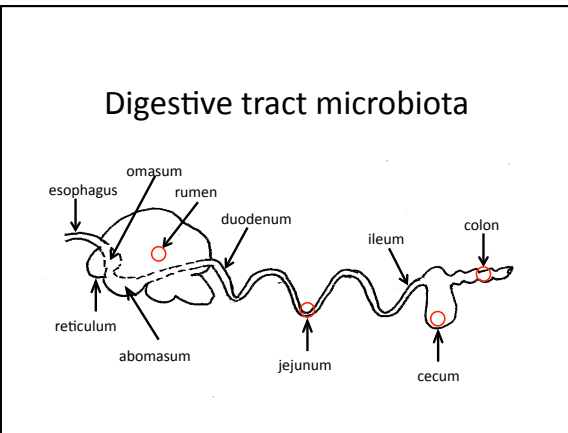
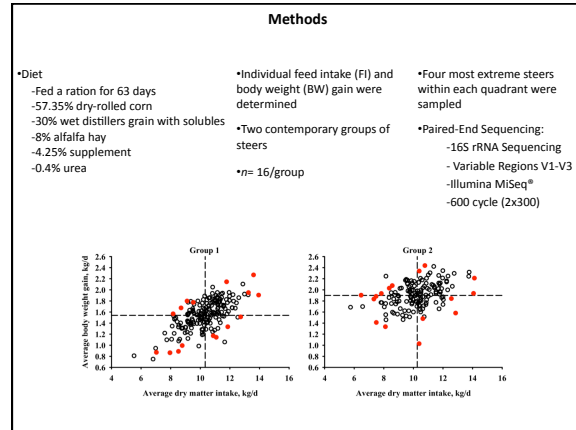
- Microbial community profiles of the rumen-reticulum, jejunum, cecum and colon of steers differing in feed efficiency
- Methane production and methanogen levels in steers that differ in residual gain

Approach

- Estimate abundance of bacteria using 16S rRNA sequences rather than culture techniques
- Investigate multiple locations in the gut associated with fermentation, digestion, and absorption

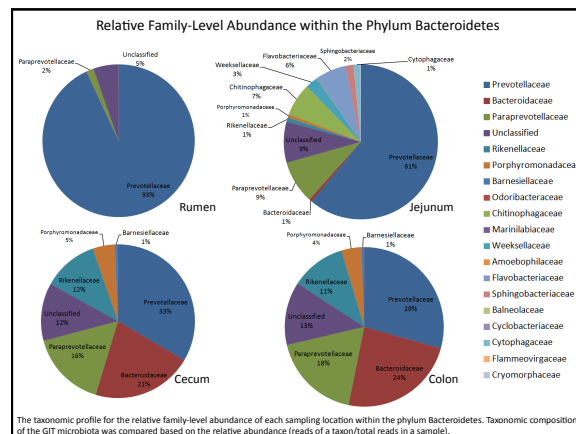
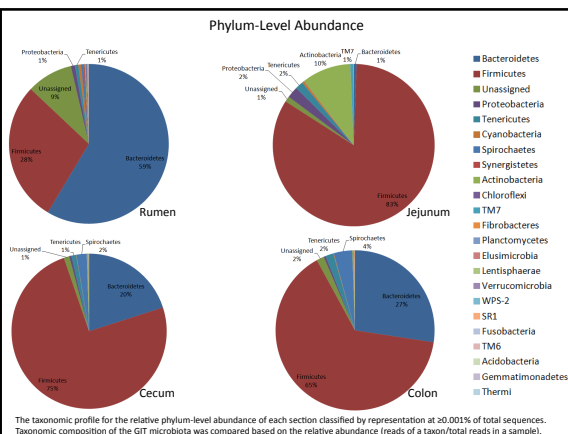
Microbial community profiles of the rumen-reticulum, jejunum, cecum and colon of steers differing in feed efficiency

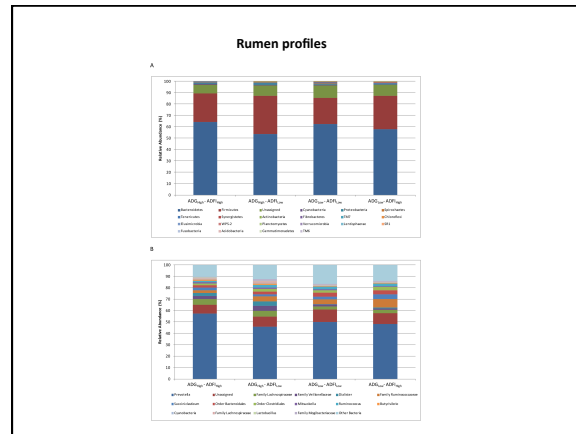
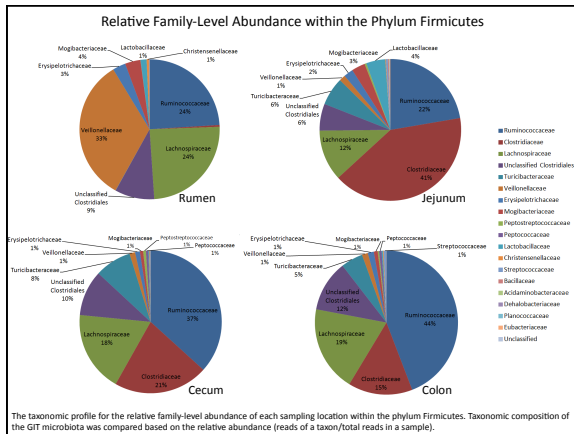
- Myer, P. R., T. P. L. Smith, J. E. Wells, L. A. Kuehn, and H. C. Freetly. 2015. Rumen microbiome from steers differing in feed efficiency. *PLoS ONE* 10:e0129174. doi:10.1371/journal.pone.0129174.
- Myer, P. R., J. E. Wells, T. P. L. Smith, L. A. Kuehn and H. C. Freetly. 2016. Microbial community profiles of the jejunum from steers differing in feed efficiency. *J. Anim. Sci.* 94:327-338. doi:10.2527/jas.2015-9839
- Myer, P. R., J. E. Wells, T. P. L. Smith, L. A. Kuehn, and H. C. Freetly. 2015. Cecum microbial communities from steers differing in feed efficiency. *J. Anim. Sci.* 93:5327-5340. doi:10.2527/jas.2015-9415.
- Myer, P. R., J. E. Wells, T. P. L. Smith, L. A. Kuehn, and H. C. Freetly. 2015. Microbial community profiles of the colon from steers differing in feed efficiency. *SpringerPlus* 4(1):1-13. doi:10.1186/s40064-015-1201-6.



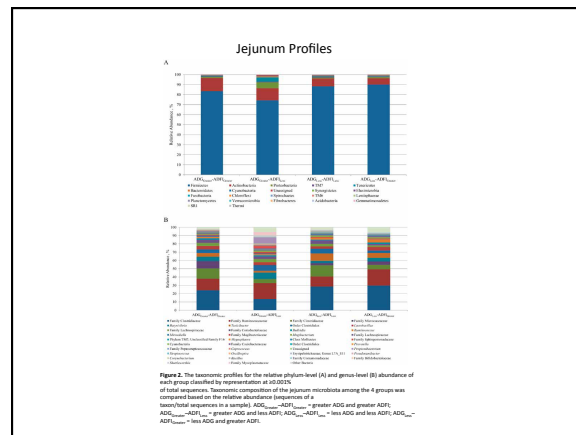
Significant Taxa and Operational Taxa Units (OTUs)

Location	Phyla	Classes	Orders	Families	Genera
Rumen	24	48	89	173	317
Jejunum	21	51	94	198	397
Cecum	18	40	75	148	225
Colon	20	46	83	152	231

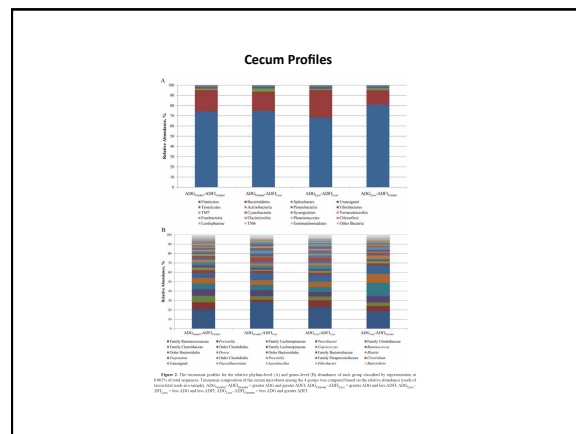




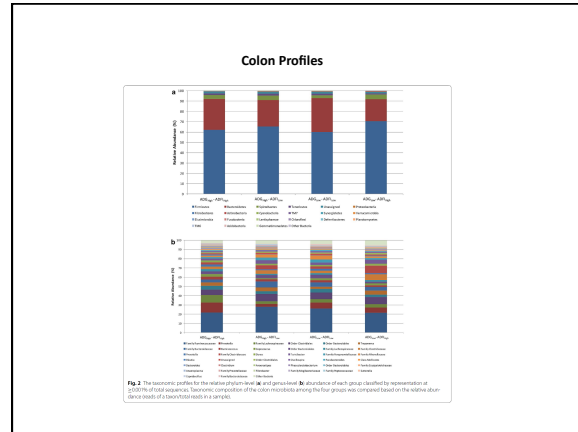
Rumen		Percentage of Total Sequences				SE	P-value	Steers with Taxon
Phylum	Sub Classification	ADG _{high} -ADFI _{high}	ADG _{high} -ADFI _{low}	ADG _{low} -ADFI _{high}	ADG _{low} -ADFI _{low}			
Firmicutes		25.24	33.43	23.00	28.99	2.64	0.036	32
Firmicutes	<i>Loctobacillus</i>	0.58	0.46	0.21	0.33	0.09	0.042	32
Firmicutes	<i>Acidaminococcus</i>	0.32	0.25	0.05	0.10	0.07	0.031	28
Firmicutes	<i>Anaerovibrio</i>	0.004	0.01	0.09	0.03	0.02	0.029	29
Firmicutes	Family Lachnospiraceae	5.11	4.85	2.67	2.81	0.67	0.020	32
Firmicutes	Family Veillonellaceae	2.69	4.25	1.67	1.28	0.59	0.006	32
Firmicutes	Family Erysipelotrichaceae; Genus RNZD	0.15	0.58	0.18	0.41	0.10	0.018	30
Proteobacteria	<i>Lysobacter</i>	4.3 X 10 ⁻⁵	4.2 X 10 ⁻¹⁰	1.3 X 10 ⁻¹³	0.001	0.0003	0.046	29
Proteobacteria	Family Helicobacteraceae	5.7 X 10 ⁻⁵	0.001	8.5 X 10 ⁻²⁰	5.7 X 10 ⁻⁵	0.0003	0.016	28
Actinobacteria	<i>Jonibacter</i>	5.7 X 10 ⁻⁵	3.4 X 10 ⁻¹⁹	5.7 X 10 ⁻⁵	0.001	0.0003	0.016	29
Actinobacteria	<i>Leucobacter</i>	2.2 X 10 ⁻⁵	0.001	3.2 X 10 ⁻¹⁹	5.8 X 10 ⁻⁴	0.0005	0.022	30
Lentisphaerae		0.013	0.008	0.052	0.09	0.02	0.033	25
Lentisphaerae	Family Victvallaceae	0.011	0.008	0.049	0.090	0.02	0.04	31



Jejunum		Percentage of Total Sequences				SE	P-value	Steers with Taxon
Classification		ADG _{high} -ADFI _{high}	ADG _{high} -ADFI _{low}	ADG _{low} -ADFI _{high}	ADG _{low} -ADFI _{low}			
Acidaminococcus		0.003	0.023	0.004	0.000	0.005	0.018	20
Ammoniphilus		9.96 X 10 ⁻⁴	4.98 X 10 ⁻⁴	5.00 X 10 ⁻⁴	3.50 X 10 ⁻⁴	0.001	0.022	27
Family Eubacteriaceae		0.000	0.000	0.000	2.5 X 10 ⁻⁴	0.001	0.049	19
Family Isospiraecae		0.000	0.000	0.000	0.003	0.001	0.039	18
Family Placoccaceae		0.011	0.001	6.8 X 10 ⁻¹⁰	0.006	0.002	0.008	19
Family Sphingomonadaceae		0.317	2.977	0.137	0.203	0.760	0.035	20
Kaistobacter		5.03 X 10 ⁻⁴	0.011	3.39 X 10 ⁻¹³	3.39 X 10 ⁻¹³	0.003	0.046	17
Lysinibacillus		0.009	0.008	0.013	0.027	0.005	0.041	30
Mycetocola		1.36 X 10 ⁻¹⁴	0.001	0.002	0.009	0.002	0.031	17
Phylum Proteobacteria		1.19	5.87	0.91	1.19	1.29	0.030	32



Classification	Percentage of Total Sequences				SE	P-value	Steers with Taxon
	ADG _{High} ⁺ ADFI _{High} ⁺	ADG _{Low} ⁻ ADFI _{Low} ⁻	ADG _{Low} ⁺ ADFI _{Low} ⁺	ADG _{High} ⁻ ADFI _{High} ⁻			
Blautia	2.00	0.63	0.87	0.50	0.35	0.036	32
Coprobacillus	0.19	0.03	0.08	0.02	0.03	0.004	28
Family Bifidobacteriaceae	5.0 × 10 ⁻⁴	0.00	0.00	0.00	1.2 × 10 ⁻⁴	0.032	16
Family Erysipelotrichaceae	0.72	0.31	0.36	0.35	0.11	0.046	31
Order Bacteroidales	2.74	2.13	1.12	3.46	0.52	0.035	31
Parabacteroides	0.81	0.35	0.34	1.51	0.26	0.027	31



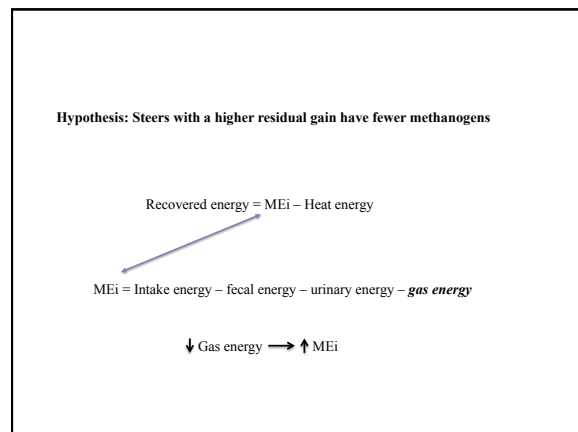
Classification	Percentage of Total Sequences				SE	P-value	Steers with Taxon
	ADG _{High} ⁺ ADFI _{High} ⁺	ADG _{Low} ⁻ ADFI _{Low} ⁻	ADG _{Low} ⁺ ADFI _{Low} ⁺	ADG _{High} ⁻ ADFI _{High} ⁻			
Anaeroplasm	0.1587	0.0591	0.0652	0.0854	0.0230	0.022	30
Cyanobacteria	0.0187	0.0103	0.0113	0.0093	0.0024	0.048	29
Faecalibacterium	0.1976	0.0276	0.0357	0.0916	0.0419	0.036	23
Family Barnesiellaceae	2.49 × 10 ⁻⁴	0.0192	0.0216	9.93 × 10 ⁻⁴	0.0063	0.047	24
Family Mogibacteriaceae	0.1013	0.1611	0.0762	0.2295	0.0358	0.031	30
Family Sphingomonadaceae	0.0012	0.0011	2.38 × 10 ⁻⁴	1.24 × 10 ⁻⁴	3.11 × 10 ⁻⁴	0.039	29
Paludibacter	1.23 × 10 ⁻⁴	4.86 × 10 ⁻⁵	0.0028	9.94 × 10 ⁻⁶	6.29 × 10 ⁻⁴	0.023	27
Prevotella	7.9657	3.1272	3.9112	3.5961	1.1520	0.026	30
Pseudobutyrvibrio	0.0187	0.0103	0.0113	0.0093	0.0024	0.048	21
Succinivibrio	0.0090	9.76 × 10 ⁻⁴	0.0020	0.0037	0.0020	0.041	29

Conclusions


- Differences in microbial populations among feed efficiency groups could be detected at the phylum or genus level
- Relative taxonomic abundance and OTU classifications indicated many significant changes in microbial populations as a function of feed efficiency
- Microbial populations that differ with feed efficiency are different in the sites of the digestive tracts
- Cannot correlate fecal microbial population abundances with microbial communities from other GIT sections

Ruminal methanogens in steers that are negative or positive for residual gain

- Freetly, H. C., A. K. Lindholm-Perry, K. E. Hales, T. M. Brown-Brandl, M. Kim, P. R. Myer, and J. E. Wells. 2015. Methane production and methanogen levels in steers that differ in residual gain. *J. Anim. Sci.* 93: 2375-2381. doi: 10.2527/jas.2014-8721.



Residual gain

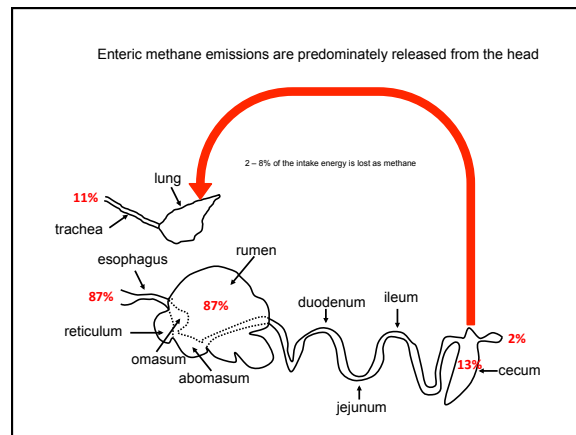
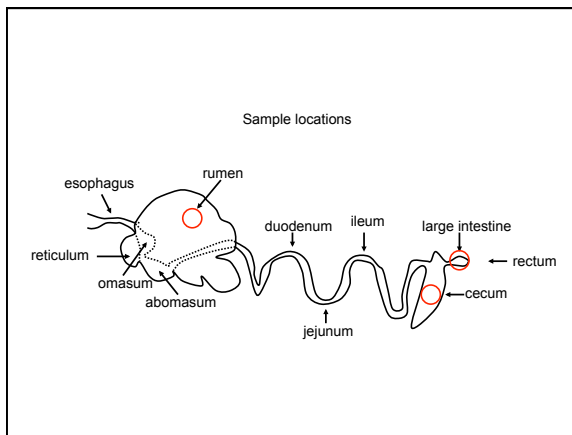
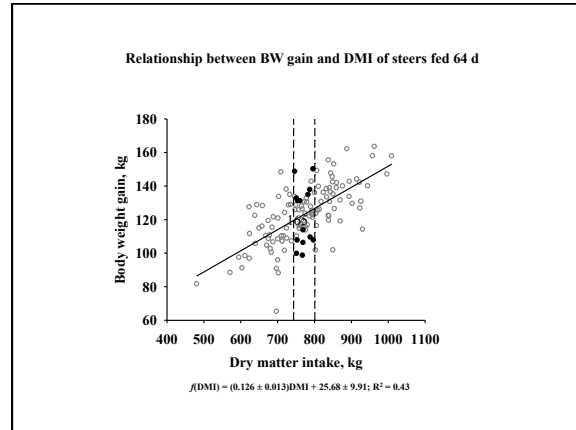


Insetec RIC Feeding System (Insetec B.V., Marknesse, The Netherlands)

Crossbred steers: 132
 - initial age = 348 ± 1 d
 - initial BW 444 ± 4 kg

Body weights determined on days 0, 1, 21, 42, 56, 62, and 63

Diet (as DM):
 82.75% rolled corn
 12.75% corn silage
 4.5% supplement (contained 0.066% monensin, 51% CP)



Background

In vitro methane production per unit dry digesta of steers differing in residual gain ($\text{mmol} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$)

Residual Gain	Rumen	Cecum	SE
Positive (n = 7)	50.81×10^{-5}	3.529×10^{-5}	7.258×10^{-5}
Negative (n = 7)	51.11×10^{-5}	3.585×10^{-5}	7.258×10^{-5}

Materials and methods

DNA was isolated and concentration was determined with NanoDrop spectrophotometer (Thermo Scientific)

- Diluted to a final concentration of 16.7 ng/μL

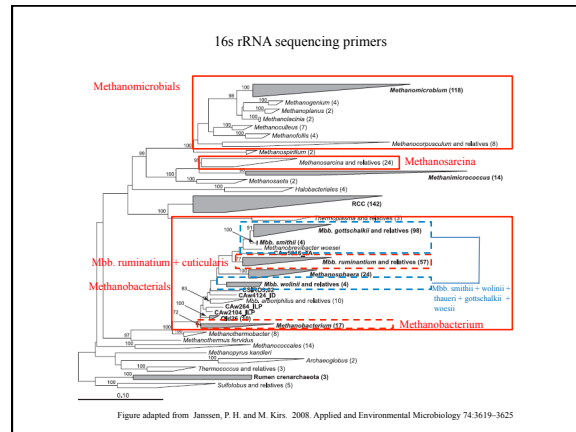
50 ng DNA loaded for real time PCR assay – Roche LightCycler 480

50 Cycles

- 95 °C for 10s
- 54 °C for 10s
- 72 °C for 10s

Standard curves were generated using plasmid DNA containing the target DNA, and diluted from the highest concentration.

16s rRNA sequencing primers				
Bacteria	-Region	Direction	Primer sequence	Base Pairs
Total methanogens	umtMet-1f	Forward	CCGGAGATGGAACCTGAGAC	165
	umtMet-1r	Reverse	CGGCTTGCCACGCTTATTC	
Methanomicrobials (Order)	MMB282f	Forward	ATCGRTACGGGTGTGGG	506
	MMB332r	Reverse	CACCTAACGRCATHTTTAC	
Methanobacteriales (Order)	MBT857f	Forward	CGWAGGGAAGCTGTAAGT	344
	MBT1196r	Reverse	TACCGTCGTCCACTCCT	
Methanosarcina (Genus)	MB1b	Forward	CGGTTGGTCAGTCCTCCGG	271
	SAR835r	Reverse	AGACACGGTCGGCCATGCTT	
Methanobacterium (Genus)	Mbt-202f	Forward	CGCTAAGGATGGATC	197
	Mbt-399r	Reverse	TAAGAGTGGCACTGGGK	
Methanobrevibacter ruminantium + Methanobrevibacter cuticularis	Mbb-g1-f	Forward	GCTAATACYGGATAGATAT	166
	A329r	Reverse	TGTCACGGTTCATCCCG	
Methanobrevibacter smithii + Mbb. thaueri + Mbb. gottschalkii + Mbb. woesei	Mbb-g2-f	Forward	GATAATCTGGATAGGCAAT	166
	A329r	Reverse	TGTCACGGTTCATCCCG	



Rumen

Copies of methanogens/ 50 ng DNA				
Residual Gain				
Positive (7)	Negative (7)	SE	P value	
8.62 × 10 ⁶	7.35 × 10 ⁶	1.10 × 10 ⁶	0.43	

Rumen

Methanogen subgroups expressed as a percentage of total methanogens

Methanogen Group	Residual Gain		SE	P value
	Positive (7)	Negative (7)		
Methanomicrobials (Order)	2.7	2.5	0.5	0.79
Methanobacteriales (Order)	41.9	43.9	2.7	0.61
Methanosarcina (Genus)	0.20	0.17	0.05	0.73
Methanobacterium (Genus)	4.3	11.5	6.5	0.44
Methanobrevibacter ruminantium + Mbb. cuticularis	8.7	8.2	1.6	0.83
Methanobrevibacter smithii + Mbb. Wolinij + Mbb. thaueri + Mbb. gottschalkii + Mbb. woesei	16.2	18.1	1.6	0.42

Cecum

Copies of methanogens/ 50 ng DNA				
Residual Gain				
Positive (7)	Negative (7)	SE	P value	
6.77 × 10 ⁶	7.26 × 10 ⁶	1.21 × 10 ⁶	0.78	

Cecum

Methanogen subgroups expressed as a percentage of total methanogens

Methanogen Group	Residual Gain		SE	P value
	Positive (7)	Negative (7)		
Methanomicrobials (Order)	0.62	0.68	0.13	0.75
Methanobacteriales (Order)	30.3	30.8	3.3	0.91
Methanosarcina (Genus)	0.04	0.04	0.01	0.81
Methanobacterium (Genus)	0.19	0.39	0.10	0.18
Methanobrevibacter ruminantium + Mbb. cuticularis	7.0	5.8	1.4	0.56
Methanobrevibacter smithii + Mbb. Wolinij + Mbb. thaueri + Mbb. gottschalkii + Mbb. woesei	10.1	11.8	1.9	0.52

Rectum

Copies of methanogens/ 50 ng DNA			
Residual Gain			
Positive (7)	Negative (7)	SE	P value
8.47×10^6	9.58×10^6	1.14×10^6	0.50

Rectum

Methanogen subgroups expressed as a percentage of total methanogens				
Methanogen Group	Residual Gain		SE	P value
	Positive (7)	Negative (7)		
Methanomicrobials (Order)	1.55	1.44	0.22	0.75
Methanobacteriales (Order)	27.7	26.6	1.8	0.69
Methanosarcina (Genus)	0.22	0.13	0.04	0.13
Methanobacterium (Genus)	0.63	1.23	0.3	0.20
Methanobrevibacter ruminantium + Mbb. cuticularis	15.2	16.5	3.5	0.79
Methanobrevibacter smithii + Mbb. Wolinii + Mbb. thaueri + Mbb. gottschalkii + Mbb. woesei	14.8	16.3	1.7	0.53

Conclusion

Our study does not support the hypothesis that steers with a higher residual gain have fewer methanogens

- High grain diet
- Similar feed intakes

- #### Other research conducted on the grant at USMARC to investigate physiological mechanisms associated with variation in feed intake and growth
- Foote, A. P., K. E. Hales, C. A. Lents, and H. C. Freetly. 2014. Association of circulating active and total ghrelin concentrations with dry matter intake, growth, and carcass characteristics of finishing beef cattle. *J. Anim. Sci.* 92:5651-5658. doi:10.2527/jas.2014-8291
 - Foote, A. P., K. E. Hales, and H. C. Freetly. 2016. Changes in acyl and total ghrelin concentrations and their association with dry matter intake, average daily gain, and feed efficiency of finishing beef steers and heifers. *Dom. Anim. Endocrinol.* doi:http://dx.doi.org/10.1016/j.domaniend.2016.05.004
 - Foote, A. P., K. E. Hales, R. G. J. Tait, E. D. Berry, C. A. W. Lents, J. E., A. K. Lindholm-Perry, and H. C. Freetly. 2016. Relationship of glucocorticoids and hematological measures with feed intake, growth, and efficiency of finishing beef cattle. *J. Anim. Sci.* 94:275-283. doi:10.2527/jas.2015-9407
 - Foote, A. P., R. G. Tait Jr, D. H. Keisler, K. E. Hales, and H. C. Freetly. 2016. Leptin concentrations in finishing beef steers and heifers and their association with dry matter intake, average daily gain, feed efficiency, and body composition. *Dom. Anim. Endocrinol.* 55:136-141. doi:http://dx.doi.org/10.1016/j.domaniend.2015.12.007

