# Feed Efficiency Phenotypes In Lambs Involve Changes In Ruminal, Colonic, And Small Intestine-Located Microbiota

Animal feed costs are one of the largest expenses for domestic livestock; those that consume less feed than would be expected based on their size, positively contribute to the economics of these livestock operations. For both beef cattle and sheep, feed efficiency is directly related to residual feed intake (RFI); the difference between how much food is actually consumed and how much was expected to be consumed based on the animal's weight<sup>2</sup>. Several studies have revealed differences in rumen-located microbes are known to form fermentative by-products (i.e. short-chain fatty acids) that provide ~70 % of the animals daily energy requirements and directly account for more than 50 % of the animals daily protein requirements<sup>3</sup>. However, how the microbiota vary in the hind gastrointestinal tract (GIT) has only been sparsely explored despite 10 - 13 % of total gut SCFAs being produced in the ruminants distal gut<sup>4</sup>. Further, how microbiota vary in the small intestine remains to be determined despite the potential for microorganisms colonizing these locations to compete for nutrients passing through this absorptive region of the GIT. Furthermore, microorganisms in all GIT locations may separately contribute to the health phenotype of the host animal. We therefore sampled the microbiota of the duodenum, jejunum, ileum, colon, and colorectally-obtained feces, in addition to the rumen of twelve lambs that, in a residual feed intake trial were found to be at either extreme of feed efficiency phenotypes to obtain a more complete picture of the gut microbiota's role in feed efficiency.

# Methods

Animal care and handling protocols were approved by the Montana State University Agricultural Animal Care and Use Committee (Protocols 2012-AA10 & 2014-AA10).

Four month old crossbred wethers (n = 65) were provided a 2-wk dietary acclimation period, and then a 47-d RFI feeding trial after vaccination for enterotoxaemia. Lambs were brought into a barn twice daily, 12 h apart, and individually penned to allow ad libitum access to an 80 %:20 % alfalfa:barley pelleted diet (Table 1) for 2 to 3 h. Feed was weighed before and after each feeding for calculating individual lamb intake. Outside of feeding periods, lambs were penned in a drylot with unlimited access to water, but no access to forage.

Double day weights were collected immediately preceding the RFI trial and averaged for BW at wk 1, 3, 4, and 6 after the adaptation period. Daily intakes for each wether were used to calculate ADG from regression coefficients of linear regression models of BW using the PROC GLM procedure of SAS (SAS Inst., Inc., Cary, NC, USA) as described by Lancaster and colleagues<sup>5</sup>. Expected feed intake (EFI) was also modeled using PROC GLM by linear regression of DMI against the modeled mid-test MBW and ADG<sup>6</sup> and was calculated for each wether as the difference between DMI and EFI.

We there with an RFI greater than (INEFFICIENT;  $RFI = 0.19 \pm 0.02$ , n = 6) and less than (EFFICIENT;  $RFI = -0.28 \pm 0.02$ , n = 6) one SD of the mean were harvested following standard industry procedures. Samples of the rumen, duodenum, jejunum, ileum, and colon, along with colorectally-obtained feces were collected and rapidly frozen in liquid nitrogen within 30 minutes of harvesting.

DNA was extracted from all samples using MoBio PowerFecal DNA isolation kits (MoBio Laboratories, Inc., Solana Beach, CA). Extracted DNA was PCR amplified using primers targeting the V3-V4 region of the 16S rRNA gene using the KAPA HotStart PCR Kit (Kapa Biosystems, Wilmington, MA). Amplified DNAs were sequenced using by Illumina Miseq. Resulting sequence data was deposited in the Sequence Read Archive and is accessible through Bioproject PRJNA354152.

Sequence data were assembled with PandaSeq<sup>7</sup> curated to remove low quality sequences using the FASTX Toolkit ( <u>http://hannonlab.cshl.edu/fastx\_toolkit/index.html</u>), UCHIME<sup>8</sup>, and mothur v 1.35<sup>9</sup>. Microbial diversity was measured as Shannon's entropy with mothur's summary.single function. Data were standardized and transformed using the Hellinger approach<sup>10</sup> and  $\beta$ -diversity measured by Bray-Curtis dissimilarity. Bray-Curtis dissimilarities were depicted by non-metric multidimensional scaling with optimization over 50,000 iterations, and differences assessed by Analysis of Similarity (ANOSIM) both using Primer  $v6^{11}$ .

One-way ANOVA was used with Bonferroni correction to assess if significant differences existed in the relative abundances of individual OTUs at each GIT location between efficient and inefficient animals. Taxonomy was inferred by RDP classifier using the SILVA database (Release 123), and further examined by BLASTn alignment to Genbank's nr/nt and curated refseq RNA databases.

## Literature Cited

- . Schnepf, R. 2012. Available: h p://digitalcommons.ilr.cornell.edu/ key\_workplace/1167
- Arthur and Herd. 2008. Revista Brasileira de Zootecnia 37:269–279 Yeoman and White. 2014. Annu. Rev. Anim. Biosci. 2:469–486.
- 4. Oh et al. 1972. J. Anim. Sci. 35:450–459.
- 5. Lancaster et al. 2009. J. Anim. Sci. 87:3887–3896. 6. Koch et al. 1963. J. Anim. Sci. 22:486–494
- . Masella et al. 2012. BMC Bioinformatics 13:31
- 8. Edgar et al. 2011. Bioinformatics 27:2194–2200.
- 9. Schloss et al. 2009. Appl. Environ. Microbiol. 75:7537–7541 10. Legendre and Gallagher. 2001. Oecologia 129:271-280
- 11. Clarke and Warwick. 2005. Nat. Environ. Res. Council.

ltem 

CP, %

NEg,

Figure 1 Alpha-Diversity of the Ruminant GIT by Location and Feed Efficiency Phenotype Strip plot showing differences in  $\alpha$ -diversity as measured using Shannon's entropy among efficient and inefficient animals

Katheryn Perea, Katharine Perz, Sarah K. Olivo, Andrew Williams, Medora Lachman, Suzanne L. Ishaq, Jennifer Thomson, Carl J. Yeoman Montana State University, Bozeman, MT, 59717, USA;

## Introduction

### Table 1. Nutrient composition (DM basis) of alfalfa:barley pelleted diet

ltem <sup>1</sup>	Value
DM, %	89.0
CP, %	20.2
ADF, %	33.2
TDN, %	64.5
NEm, mcal/lb	0.65
NEg, mcal/lb	0.37

<sup>1</sup>Average of samples taken from each batch (n=6) of feed used

Microbiota Vary With Gut Anatomy But No Large Scale Restructuring Is Seen With Feed **Efficiency Phenotypes** 



# more similar.

### Microbiota Are Typically More Similar to Microbiota Of Their Nearest Distal Gut Regions **Than Other Gut Locations**

different GIT regions.

	Colon	Duodenum	Feces	Ileum	Jejunum	Rumen
Colon						
Duodenum	0.987**					
Feces	0.296	0.974**				
Ileum	0.889*	0.602**	0.872**			
Jejunum	0.944**	0.291**	0.931**	0.113		
Rumen	1**	0.365*	1**	0.815**	0.652**	

Analysis of Similarities (ANOSIM) R values indicate how dissimilar two microbial communities are from one another and range from 0 (identical) to 1 (no common features). Significance is indicated as \* *P*<0.005, \*\**P*=0.001

### Microbial Diversity Is Highest In The Colon But **Does Not Vary With Feed Efficiency Phenotypes**



# **Results & Conclusions**

Figure 2 Non-metric multidimensional scaling of Bray-Curtis dissimilarity NMDS shows the relationship of microbiota among samples collected from differing GIT locations of all animals and stratified by feed efficiency phenotype. Each symbol represents a whole microbial community and the distance between symbols indicate their compositional similarities, with closer symbols being

Table 2. ANOSIM Relationships among microbiota of

### **Individual Microbes Related To Fibrolysis In The Rumen** and Colon And Those Related To Health In The Small **Intestine Differ With Feed Efficiency**

 
 Table 3. Microbes Whose Presence or Relative Abundance Was
**Greater in Highly Efficient Phenotypes** 

OTU	Taxonomy <sup>a</sup>	n (Efficient / Inefficient)	- corrected P-value	
	Rumen			
3	Ruminococcaceae family	6/6	0.04	
	(Uncultured Rumen Bacterium, 99%/ Intestinimonas butyriciproducens 93%)	070	0.04	
4	Unclassified bacterium	6 / 6	0.04	
	(Uncultured Pig GIT Bacterium, 98%/ Saccharofermentans acetigenes 89%)			
32	Saccharofermentans spp.	6 / 6	0.06	
	(Uncultured Rumen Bacterium, 99%/ Saccharofermentans acetigenes 94%)			
	Jejunum			
106	Bifidobacteriaceae family	5 / 0	0.02	
	(Uncultured Cattle Abomasal Bacterium, 99%/ Bifidobacterium saeculare, 89%)			
Colon				
10	Fibrobacter spp.	6 / 6	0.06	
	(Uncultured Rumen Bacterium, 99%/ Fibrobacter succinogenes, 99%)			
44	Rikenellaceae family	6 / 5	0.02	
	(Uncultured Rumen Bacterium, 98%/ Marinilabilia salmonicolor, 85%)			
Feces				
3	Ruminococcaceae family	6 / 6	0.07	
	(Uncultured Rumen Bacterium, 99%/ Intestinimonas butyriciproducens 93 %)			
14	Christensenellaceae family	6/6	0.05	
	(Uncultured Rumen Bacterium, 99%/ Alkalibaculum bacchi, 88%)	070		

### Table 4. Microbes Whose Presence or Relative Abundance Was **Greater in Inefficient Phenotypes**

ΟΤυ	Taxonomic Classification	n (Efficient / Inefficient)	Bonferoni - corrected P-value	
	Rumen			
56	Lachnospiraceae family (Uncultured Rumen Bacterium, 99%/ <i>Clostridium xylanolyticum</i> , 96%)	6 / 6	0.04	
77	Succinivibrio spp. (Uncultured Human Stool Bacterium, 98%/ Succinivibrio dextrinosolvens, 97%)	6 / 6	0.05	
110	UCT N177 order (Uncultured Oragutan Fecal Bacterium, 96%/ Oxalobacter vibriformis, 92%)	4 / 6	0.04	
Duodenum				
37	Proteobacteria phyla (Uncultured Sheep Fecal Bacterium, 99%/ Devosia lucknowensis, 85%)	5 / 6	0.05	
Colon				
42	Alloprevotella spp. (Uncultured Cattle Colon Bacterium, 98%/ Alloprevotella rava, 93%)	6 / 6	0.06	
Feces				
8	Gastranaerophilales order (Uncultured Fermenter Bacterium, 99%/ Vampirovibrio chlorellavorus, 87 %)	6 / 6	0.05	
30	<i>Thalassospira</i> spp. (Uncultured Cattle Fecal Bacterium/ <i>Insolitispirillum peregrinum</i> 87%)	6 / 6	0.02	
33	Alphaproteobacteria class (Uncultured Cattle Fecal Bacterium, 94%/ <i>Pedomicrobium ferrugineum</i> , 87 %)	6 / 6	0.03	
42	Alloprevotella spp. (Uncultured Cattle Colon Bacterium, 98%/ Alloprevotella rava, 93 %)	5 / 6	0.04	

**Our findings show biospatial delineations of microbiota** throughout the GIT and suggest that feed efficiency extends beyond the rumen, and involves increases in both rumen-, and colon-located fibrolytic taxa, increases in bifidobacterial species in the small intestine, and reductions in small intestine and distal GIT-located Proteobacteria.

# Acknowledgements

The data herein is currently described in an in press paper with the Journal of Animal Sciences (Perea et al. *In press* JAS)



MONTANA INBRE USDA M MONTANA STATE UNIVERSIT **NIFA** MONTANA AGRICULTURA

EXPERIMENT STATIO