

Feed efficiency phenotypes in lambs involve changes in ruminal, colonic, and small-intestine-located microbiota¹

K. Perea, K. Perz, S. K. Olivo, A. Williams, M. Lachman, S. L. Ishaq, J. Thomson,^{2,3} and C. J. Yeoman^{2,3}

Department of Animal and Range Science, Montana State University, Bozeman 59717

ABSTRACT: Several studies have revealed differences in rumen-located microbes between greatly efficient and inefficient animals; however, how the microbiota vary in the hind gastrointestinal tract (GIT) has only been sparsely explored and how they vary in the small intestine remains to be determined. We therefore sampled the microbiota of the duodenum, jejunum, ileum, colon, and colorectally-obtained feces, in addition to the rumen of 12 lambs that, in a residual feed intake trial, were found to be at either extreme of feed efficiency phenotypes. The 16S rRNA gene (V3-V4 region) profiles of all samples were analyzed and revealed unique microbiota in all GIT locations except the jejunum and ileum (ANOSIM $R > 0.2$, $P < 0.001$). Measures of β -diversity revealed greater dissimilarity between more anatomically distant GIT locations (e.g., Rumen–Duodenum, ANOSIM $R = 0.365$, $P < 0.001$; Rumen–Colon, ANOSIM $R = 1$, $P < 0.001$) with the nearest distal

region typically more similar than the nearest proximal location. The relative abundances of 13 operational taxonomic units (OTUs) from the duodenum, jejunum, colon, and feces, as well as the rumen, differed between efficient and inefficient animals (Bonferroni corrected, $P < 0.05$), while another 2 OTUs trended toward significance. These OTUs were classified as taxa with known roles in fibrolysis (Fibrobacteres, Ruminococcaceae, and Saccharofermentans) and others that are commonly associated with health (Bifidobacteriaceae, and Christensenellaceae) and dysbiosis (Proteobacteria). Our findings show biospatial delineations of microbiota throughout the GIT and suggest that feed efficiency extends beyond the rumen, transcending these regions, 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.

Key words: feed efficiency, gastrointestinal tract, gut biospatiality, lamb, microbiota, residual feed intake

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INTRODUCTION

Animal feed costs are one of the largest expenses for domestic livestock operations (Barnard, 1969;

Miller et al., 2001; Schnepf, 2012). Therefore, more efficient livestock, those that consume less feed than would be expected based on their size, positively contribute to the economics of these livestock operations. Reductions in feed required to sustain an operation and the land necessary to produce that feed amount to potential increases in economic and environmental sustainability. By better understanding the factors that control and guide feed efficiency, resource distribution and profits can be maximized. 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 (Arthur and Herd, 2008). More feed-efficient animals have been found to exhibit various phenotypical features, including reduced subcutaneous fat (Richardson et al., 1998),

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³Corresponding authors: jennifer.thomson@montana.edu; carl.yeoman@montana.edu

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reduced heat and methane emissions (Nkrumah et al., 2006), and greater DM and CP digestibility (Nkrumah et al., 2006). Diet and digestibility have been estimated to account for 19% of the variation in feed efficiency phenotypes, while the remaining 81% remains unaccounted for (Herd and Arthur, 2009).

For ruminant livestock species, the GIT microbiota are indispensable to the nutritive function of the animal. The typically low-quality feed consumed by these animals principally comprises host-indigestible carbohydrates in the form of structural polysaccharides and are often low in protein. Consequently, ruminants rely on fermentative byproducts (i.e., short-chain fatty acids) produced by their GIT microbes for ~70% of their daily energy requirements and the microbes themselves for more than 50% of their daily protein requirements (Yeoman and White, 2014). It is therefore reasonable to hypothesize that the GIT microbiota of ruminant livestock make significant contributions to feed efficiency phenotypes. In this context, the pregastric rumen has logically received much attention due to its immense size and importance, representing ~80% of total GIT mass and serving as the location where > 85% of the total SCFAs are produced in a ruminants GIT (Oh et al., 1972). Studies have shown that rumen-colonizing microbes do covary with feed efficiency phenotypes (Guan et al., 2008), and these changes correspond to the concentrations of rumen short-chain fatty acid concentrations, particularly butyrate and valerate (Guan et al., 2008). Specific ruminal taxa that have been associated with feed efficiency include increases in *Butyrivibrio*, and *Succinivibrio* species, incompletely classified bacteria of the Ruminococcaceae family, and Bacteroidales order, along with decreases in *Lactobacillus*, *Robinsoniella*, *Selenomonas*, *Succiniclaticum* species or OTUs (Hernandez-Sanabria et al., 2012; McCann et al., 2014; Myer et al., 2015b). Members of the *Prevotella* genus, typically observed as the most abundant genera of the rumen, have been shown to have both greater and lesser relative abundances in feed efficient animals (McCann et al., 2014; Myer et al., 2015b), including in the same study (Myer et al., 2015b), perhaps suggesting that restructuring of subgenus level populations is important to feed efficiency. These may include unresolved species-level changes or even subspecies level variation, as has previously been shown to be important among other fibrolytic ruminal taxa (Brulc et al., 2011). Despite these findings, little information exists on the differences in more distal colonic regions of the GIT (Myer et al., 2015a) and it is heretofore unknown if changes also occur in regions of the small intestine. Yet, these regions may be of great importance. The ruminant colon, for instance, maintains a similar microbial density as is observed among monogastric animals that still receive nutritive benefit even when they consume higher-quality feeds (Yeoman

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

Item ¹	Value
DM, %	89.0
CP, %	20.2
ADF, %	33.2
TDN, %	64.5
NEm, mcal/lb	0.65
NEg, mcal/lb	0.37

¹Average of samples taken from each batch ($n = 6$) of feed used

and White, 2014). Evidence already exists to show that energetically-important microbial products, including VFA (10%–13% of total GIT VFA) are produced in the ruminant distal gut (Oh et al., 1972). Microorganisms colonizing the small intestine may 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 set out to determine if greatly efficient and greatly inefficient lambs exhibited differences in measures of microbial richness, α -diversity, or composition (β -diversity), or if specific taxa varied in any of five different GIT locations, including the rumen, duodenum, jejunum, ileum, and colon as well as the feces.

MATERIALS AND METHODS

Experimental Design

Animal care and handling protocols were approved by the Montana State University Agricultural Animal Care and Use Committee (Protocols 2012-AA10 and 2014-AA10). This experiment was conducted at the Montana State University Bozeman Agricultural Research and Teaching Farm (BARTF). 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. Body weights were recorded for each lamb on 2 consecutive days 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. Average daily gains (kg/d) of individual wethers were modeled by linear regression of BW using the PROC GLM procedure of SAS (SAS Inst. Inc., Cary, NC). The regression coefficients were

used to compute the ADG, initial and final BW, and mid-test metabolic BW (**MBW**) as described by Lancaster et al. (2009). Expected feed intake (**EFI**) was modeled using PROC GLM by linear regression of DMI against the modeled midtest MBW and ADG (Koch et al., 1963). The model used to estimate EFI was

$$Y_i = \beta_0 + \beta_1 \text{ADG}_i + \beta_2 \text{mid-test MBW}_i + \varepsilon_i,$$

where Y_i is the DMI of the ewe, β_0 is the regression intercept, β_1 is the partial regression coefficient of DMI on modeled ADG, β_2 is the partial regression coefficient of DMI modeled on midtest MBW, and ε_i is the residual error in the DMI of the wethers. Residual feed intake was calculated for each wether as the difference between DMI and EFI. Wethers with an RFI greater than (**INEFFICIENT**; $\text{RFI} = 0.19 \pm 0.02$, $n = 6$) and less than (**EFFICIENT**; $\text{RFI} = -0.28 \pm 0.02$, $n = 6$) 1 SD of the mean of the 65 wethers were harvested following standard industry procedures. Initial and final live weights, and ADG did not differ between the 2 groups. Samples of the rumen, duodenum, jejunum, ileum, and colon, along with coloretally-obtained feces, were collected and rapidly frozen in liquid nitrogen within 30 min of harvesting. Samples were subsequently transferred to -80°C freezer storage on returning to the lab where they remained until processing (within 2 mo).

DNA Extraction, Preparation, and Sequencing

Metagenomic DNA was extracted from all samples using MoBio PowerFecal DNA isolation kits (MoBio Laboratories, Inc., Solana Beach, CA). Manufacturer's protocols for DNA extractions were strictly adhered to and incorporated a 1-min bead homogenization using 0.7-mm garnet beads supplied with the MoBio kit. Bead homogenization occurred after the addition of solution C1 and subsequent heating as described for the experienced user protocol at steps 4–6 and was performed in a Mini-Beadbeater-96 (Biospec products, Bartlesville, OK) at 2400 oscillations/min. Extracted DNA was amplified by 25 cycles of PCR using the KAPA HotStart PCR Kit (Kapa Biosystems, Wilmington, MA): 10 ML Kappa HotStart Mastermix, 6 ML molecular-grade water, 1 ML of each forward and reverse primer, and 2 ML sample DNA. The Hot Start protocol was as follows: 95°C for 3 min; followed by 25 cycles of denaturation at 98°C for 20 s, annealing at 52°C for 30 s, and elongation at 72°C for 45 s. Primers were as previously described by Swartz et al. (2014) and targeted the V3-V4 region of the 16S rRNA gene. All amplicons were then examined for quantity and quality on an Agilent 2200 tape station using D1K Screentapes, 3 μL of D1K

buffer, and 1 μL of PCR product in each tube before being pooled at equimolar concentrations. The pooled DNAs were quantified by qPCR using a BioRad CFX-96 C1000 thermocycler and strictly adhering to the protocols of the Kapa Illumina library quantification kit (Kapa Biosystems, Wilmington, MA). The sample pool was then diluted to 15 pM, spiked with 10% of the PhiX sequencing control V3 (Illumina, San Diego, CA) and loaded on to the Illumina Miseq. Samples were sequenced using V3 chemistry to give 2x300nt reads. Resulting sequence data were deposited in the Sequence Read Archive and are accessible through Bioproject PRJNA354152.

Sequence Processing

Resulting paired-ended V3-V4 16s rRNA gene reads were assembled with PandaSeq (Masella et al., 2012) using default settings. Assembled sequences were then curated to remove low-quality ($< Q30$) sequences using the FASTX Toolkit (http://hannonlab.cshl.edu/fastx_toolkit/index.html), sequences shorter than 200 nt, with homopolymeric tracts longer than 8, or exhibiting an ambiguous base call using mothur v 1.35 (Schloss et al., 2009), and chimeric sequences using mothur's implementation of UCHIME (Edgar et al., 2011). Sequences were clustered using mothur's average neighbor approach with a clustering diameter of 0.03.

Data Analyses

Measures of coverage, observed (**SOBS**) and estimated (Chao1) OTU richness, and diversity (Shannon's and Simpson's) were collected using mothur's various calculator functions. Data were standardized and transformed using the Hellinger approach (Legendre and Gallagher, 2001) and β -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**). Bray-Curtis dissimilarities, non-metric multidimensional plots, and ANOSIM were performed using Primer 6 (Clarke and Warwick, 2005).

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. For each OTU whose relative abundance was found to significantly differ between efficient and inefficient animals, taxonomy was inferred by mothur's implementation of the RDP classifier using the SILVA database (Release 123), and further examined by BLASTn alignment of the most representative sequence (by

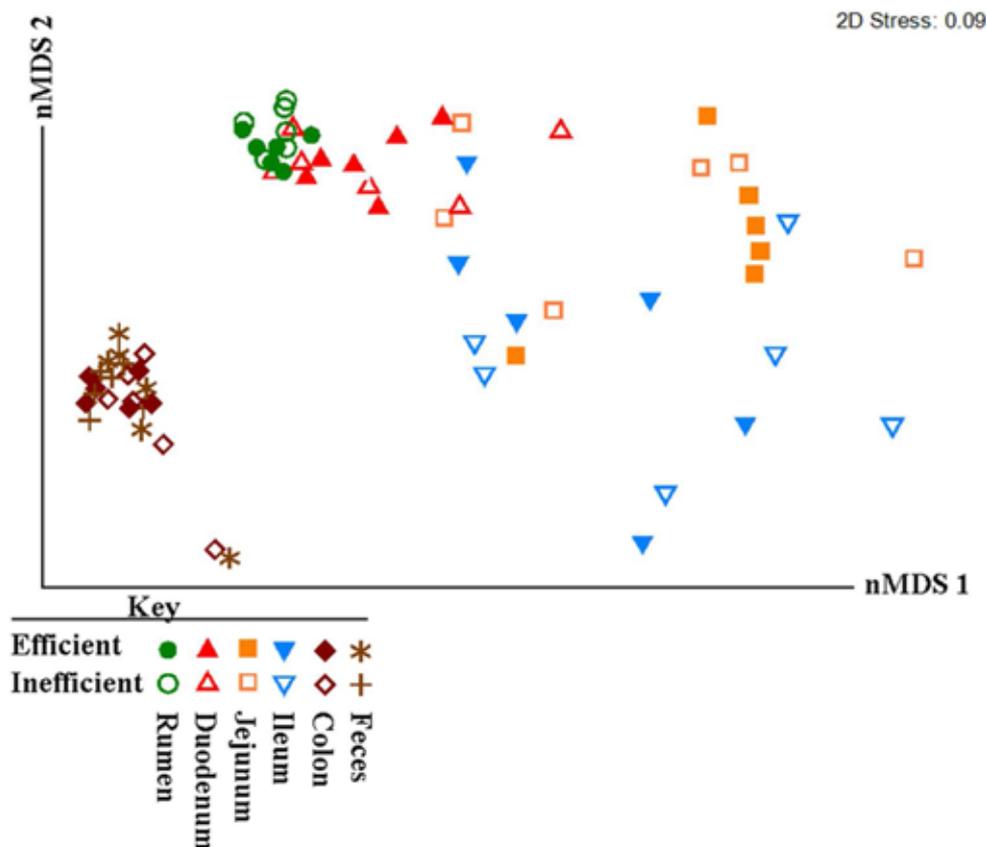


Figure 1. The biospatiality of the rumen GIT microbiota. Non-metric multidimensional scaling of Bray-Curtis dissimilarity showing the relationship of microbiota among samples collected from differing GIT locations of all animals and stratified by feed efficiency phenotype.

mothur's definition this is the sequence that is closest to all other sequences clustered into the OTU) to Genbank's nr/nt database and the refseq_RNA database. RDP classifier-based classifications were determined to be supported if they achieved bootstrapping support of > 80% and BLASTn if coverage was > 98%, nucleotide identity was > 85%, and E-values were < 1e-100. The best BLASTn match in each database is reported. The best matches in the nr/nt database were in all instances of uncultured taxa; in some instances, the origin of these sequences was not part of the name, these matches were further investigated to determine where the sequence originated from, and this was included in the description.

RESULTS

Deep Coverage of the Lamb GIT Microbiota

A total of 226,642 high-quality reads (average 18,888 reads per sample) were obtained, and samples were randomly subsampled to 12,000 reads for direct comparison. Good's nonparametric coverage estimates indicated we had extensively surveyed the microbiota in each location of the gastrointestinal tract (GIT; average 0.99 ± 0.01 , range 0.94- > 0.99).

Biospatiality of the Lamb GIT Microbiota

Non-metric multidimensional scaling (nMDS) was used to visualize Bray-Curtis dissimilarities among all samples (Fig. 1). Overall, samples clustered by GIT location, with their degrees of similarity reflecting GIT anatomy (Table 2). While rumen samples exhibited partial overlap with those obtained from the duodenum, which may reflect a sink:source relationship, ANOSIM indicated an overall difference in the microbial composition in the 2 GIT locations (ANOSIM $R = 0.353$ $P = 0.001$). The rumen microbiota was then progressively less similar to more distal regions of the GIT—the jejunum ($R = 0.652$, $P = 0.001$), ileum ($R = 0.815$, $P = 0.001$), colon ($R = 1$, $P = 0.001$), and feces ($R = 1$, $P = 0.001$). Similarly, the

Table 2. Relationships among microbiota of 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**	

* $P < 0.005$, ** $P = 0.001$

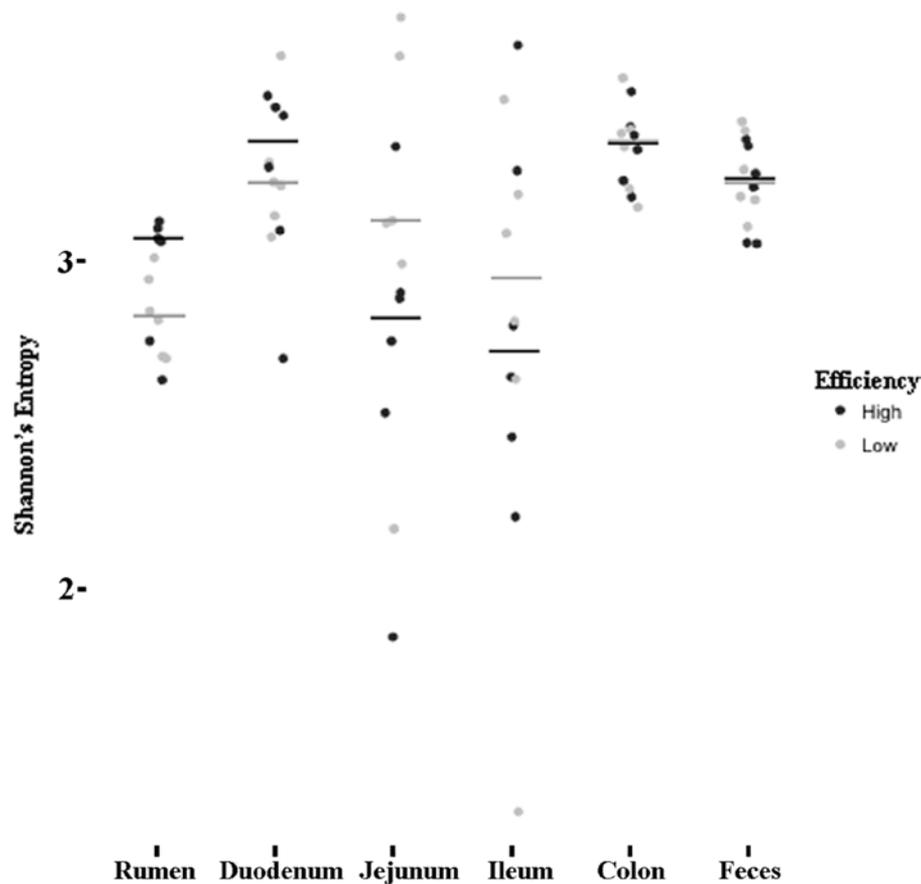


Figure 2. Alpha-diversity of the ruminant GIT by location and feed efficiency phenotype. Strip plot showing differences in α -diversity as measured using Shannon's entropy among efficient and inefficient animals.

duodenum was most similar to the jejunum, the jejunum most similar to the ileum (and vice versa), and the colon most similar to feces (Table 2). Each of these regions was then progressively less similar to the more proximal and distal regions of the GIT (Table 2). With the exception of the ileum, the nearest distal region was always more similar than the nearest proximal region, perhaps reflecting the primarily unidirectional flow of substrate and microbiota through the GIT.

Ruminal (average Bray-Curtis dissimilarity = 0.17), colonic (0.21), and fecal (0.22) samples displayed smaller between-individual variation, as is visually evident from the more tightly-formed clusters seen in Fig. 1, than samples of the small intestine (duodenum, 0.33; jejunum, 0.41; and ileum, 0.41). Similarly, substantial variation was seen among animals in the α -diversity of ileum and jejunum samples (Fig. 2). Both measures of Shannon's entropy and the Simpson's diversity index (Simpson's data not shown) supported greater overall α -diversity in the colon than other regions of the GIT ($P < 0.05$; Fig. 2).

Relationship to Feed Efficiency

Efficient and inefficient animals did not significantly differ from one another in measures of α -diversity at any

of the GIT locations tested (Fig. 2). Similarly, the overall composition (Bray-Curtis β -diversity) of the microbiota observed in each GIT location did not differ between efficient and inefficient animals (ANOSIM $R < 0.2$, $p > 0.1$; Fig. 1). However, differences were seen for the relative abundances of 13 OTUs between efficient and inefficient animals from the rumen, duodenum, jejunum, colon, and feces. Specifically, 5 OTUs had a greater relative abundance in efficient animals ($P < 0.05$; Table 3) and 8 had a greater relative abundance in inefficient animals ($P < 0.05$; Table 4). Two further OTUs also trended toward being of significantly greater relative abundance in efficient animals ($P < 0.1$; Table 3). Taxonomic assessment of these OTUs, revealed all but 1 (a chemostat fermenter) OTU to be most similar to GIT-located organisms, and largely from cattle or sheep rumen ($n = 7$), abomasum ($n = 1$), colon ($n = 1$), or feces ($n = 3$). Of the 15 OTUs found to differ in their relative abundances between efficient and inefficient animals, 10 were present in all animals and differed only in their relative abundances. OTU 37 was detected in all but a single efficient animal. Similarly, OUT 42 was present in the colon of all animals but not detected in the feces of 1 efficient animal. OTU 44 was detected in all but a single inefficient animal, and OTU 110 was detected in all but 2 efficient

Table 3. Identity of OTUs found to have greater relative abundances in efficient vs. inefficient lambs

OTU	Taxonomy ¹	<i>n</i> (Efficient/Inefficient)	Bonferroni-corrected <i>P</i> -value
Rumen			
3	Ruminococcaceae family (Uncultured Rumen Bacterium, 99%/ <i>Intestinimonas butyriciproducens</i> 93%)	6/6	0.04
4	Unclassified bacterium (Uncultured Pig GIT Bacterium, 98%/ <i>Saccharofermentans acetigenes</i> 89%)	6/6	0.04
32	Saccharofermentans spp. (Uncultured Rumen Bacterium, 99%/ <i>Saccharofermentans acetigenes</i> 94%)	6/6	0.06
Jejunum			
106	Bifidobacteriaceae family (Uncultured Cattle Abomasal Bacterium, 99%/ <i>Bifidobacterium saeculare</i> , 89%)	5/0	0.02
Colon			
10	Fibrobacter spp. (Uncultured Rumen Bacterium, 99%/ <i>Fibrobacter succinogenes</i> , 99%)	6/6	0.06
44	Rikenellaceae family (Uncultured Rumen Bacterium, 98%/ <i>Marinilabilia salmonicolor</i> , 85%)	6/5	0.02
Feces			
3	Ruminococcaceae family (Uncultured Rumen Bacterium, 99%/ <i>Intestinimonas butyriciproducens</i> 93%)	6/6	0.07
14	Christensenellaceae family (Uncultured Rumen Bacterium, 99%/ <i>Alkalibaculum bacchi</i> , 88%)	6/6	0.05

¹Taxonomy as inferred by RDP-classification. Best BLASTn matches from the nr/nt and refseq_rna databases are also shown in parentheses along with their % nucleotide identities.

animals. OTU 106, a jejunal-located Bifidobacteriaceae family member most similar to *Bifidobacterium saeculare*, was uniquely observed in only efficient animals.

DISCUSSION

Consistent with previous studies, we observed no differences in measures of α -diversity between feed efficiency phenotypes (McCann et al., 2014; Myer et al., 2015b) and no overall differences in β -diversity (Myer et al., 2015b). Instead, feed efficiency phenotypes were characterized by changes in individual OTUs representing taxa associated with fibrolysis, GIT health, and dysbiosis. This implies that efficiency status does not depend on larger-scale restructuring of the entire microbial community, but is instead dependent on differences in a handful of key taxa. Differences we observed in some ruminal taxa, specifically *Succinivibrio* spp. and a Ruminococcaceae family member (in our case, we found this OTU to be most similar to *Intestinimonas butyriciproducens*), were consistent with those previously described in studies of steers (Hernandez-Sanabria et al., 2012) and Brahman bulls, respectively (McCann et al., 2014). OTUs identified as fibrolytic taxa, including the cellulolytic *Fibrobacter succinogenes*; the at least amylolytic *Saccharofermentans*; the fructo- and galactooligosaccharide fermenting Bifidobacteriaceae; and the α -arabinosidase, β -galactosidase, and β -glucosidase-producing Christensenellaceae (Christensenellaceae have been shown to degrade chicory and chitosan) were each found to have greater relative abundances in dif-

ferent regions of the GIT (Morotomi et al., 2012; Li et al., 2016). OTU 3 was identified as a Ruminococcaceae family member; this bacterial family comprises known fibrolytic members, including two major ruminal cellulolytic species, *R. albus* and *R. flavefaciens*. The closest match to OTU 3, *Intestinimonas butyriciproducens*, has been shown to degrade dextrans, turin-, and maltotriose (Klaring et al., 2013). Interestingly, this OTU was found to have a greater relative abundance in both the rumen and feces of efficient lambs when compared to inefficient lambs, perhaps indicating an important role in both the rumen and hind-GIT. Consistent with a previous observation of increased concentrations of ruminal butyrate, *Intestinimonas butyriciproducens* is a known butyrate producer (Klaring et al., 2013), as are Christensenellaceae (Morotomi et al., 2012). However, there did not appear to be a consistent enrichment of butyrate producers among efficient animals. For example, we observed a reduction of OTU 56, identified as a Lachnospiraceae family member (a family that includes the often-prevalent *Butyrivibrio-Pseudobutyrvibrio* clade of bacteria and other known butyrate producers), and no significant differences across OTUs that could be further classified within the *Butyrivibrio* or *Pseudobutyrvibrio* genera. Similarly, we observed a general reduction in succinate producers, including OTUs that were identified as *Succinivibrio* and *Alloprevotella* spp. in both the rumen and colon. However, this observation was also not consistent with the colonic enrichment of an OTU that was > 99% identical to *Fibrobacter succinogenes*, a taxon known to

Table 4. Identity of OTUs found to have greater relative abundances in inefficient vs. efficient lambs

OTU	Taxonomic classification ¹	n (Efficient/Inefficient)	Bonferroni-corrected P-value
Rumen			
56	Lachnospiraceae family (Uncultured Rumen Bacterium, 99%/Clostridium xylanolyticum, 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 vibriiformis, 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	Thalassospira spp. (Uncultured Cattle Fecal Bacterium/Insolitispirillum peregrinum 87%)	6/6	0.02
33	Alphaproteobacteria class (Uncultured Cattle Fecal Bacterium, 94%/Pedomicrobium ferrugineum, 87%)	6/6	0.03
42	Alloprevotella spp. (Uncultured Cattle Colon Bacterium, 98%/Alloprevotella rava, 93%)	5/6	0.04

¹Taxonomy as inferred by RDP-classification. Best BLASTn matches from the nr/nt and refseq_rna databases are also shown in parentheses along with their % nucleotide identities.

produce succinate as a major end product (Stewart and Flint, 1989). The other major endproduct of *F. succinogenes* is acetate (Stewart and Flint, 1989), and consistently, OTUs identified as taxa (i.e., *Saccharofermentans*, *Bifidobacteriaceae*) known to produce acetate as major endproducts had greater relative abundances in the rumen, small intestine, and distal GIT.

Intestinimonas butyriciproducens is also known as a lactate utilizer (Klaring et al., 2013), and if this is a feature of OTU 3, this OTU may serve to keep this potentially hazardous fermentative product (Russell and Rychlik, 2001) at sustainable levels. Other taxa further suggest that a healthy microbiome is an important feature of feed efficiency. Greater relative abundances of Proteobacteria (OTUs 30, 33, and 37) in the small intestine and distal GIT may reflect dysbioses of these GIT regions among less efficient animals. Increased relative abundances of Proteobacteria is a common GIT feature of unhealthy groups, including those suffering from inflammatory bowel disease (Mukhopadhyaya et al., 2012), diarrhea (Suchodolski et al., 2015), as well as obese individuals (Zhao, 2013), where endotoxin of the well-known Proteobacterial member, *E. coli*, has been shown to affect subcutaneous adiposity (Cani et al., 2008). Conversely, the enrichment of a Bifidobacteriaceae OTU in the small intestine of efficient animals may be a sign of health (Mitsuoka, 1990). Similarly, Christensenellaceae, recently found to be one of the more inheritable taxa present forming co-occurrence relationships with various bacte-

ria and methanogenic archaea in the GITs of both humans and mice, has been broadly associated with health and a better functioning digestive system (Goodrich et al., 2014). For example, Christensenellaceae have been shown to have a greater relative abundance in fecal samples of healthy human individuals compared to those with inflammatory bowel disease (Goodrich et al., 2014), in pigs with lesser amounts of *Escherichia coli* shedding (Jenkins et al., 2015), and to be anticorrelated with loose stool (Tigchelaar et al., 2016). Furthermore, the addition of *Christensenella minuta* to human fecal material used to inoculate germ-free mice was found to reduce adiposity (Goodrich et al., 2014), an observation that is consistent with a previous work showing that efficient animals had reduced subcutaneous fat (Richardson et al., 1998).

Collectively, our results indicate differences in the feed efficiency phenotypes of lambs involving changes in the relative representations of a subset of microbial taxa found throughout the GIT and with roles in fibrolysis, health, and dysbiosis.

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