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

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Introduction

Animal feed costs are one of the largest expenses for domestic livestock operations. Therefore, more efficient livestock; those that consume less feed than would be expected based on their size, contribute positively 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. Several studies have revealed differences in rumen-located microbes between highly efficient and inefficient animals. Therein 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. However, how the microbiota in the hind gastrointestinal tract (GIT) has only been sparingly explored despite 10 – 13 % of total gut SCFAs being produced in the ruminants distal gut. 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. Therefore we sampled the microbiota of the duodenum, jejunum, ileum, colon, and colostrally-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 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-week dietary acclimation period, and then a 42-d RFI feeding trial after vacuums for enterostomies. Lambs were brought into a barn twice daily, 12 h apart, and individually penned to allow ad libitum access to an 8% NDF alfalfa/barley pelleted diet (Table 3) 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 stall with unilateral 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. Expected feed intake (RFI) was also modeled using PROC GLM with linear regression of DMI against the BW of the lamb. RFI = -0.28 (R2 = 0.33) was also modeled using PROC GLM by linear regression of DMI against the difference between DMI and ADG modeled mid-test MBW and ADG calculated for each wether as the difference between DMI and ADG.

Wethers with an RFI greater than (INEFFICIENT; RFI = 0.19 ± 0.02, n = 6) and less than (EFFICIENT; RFI = 0.19 ± 0.02, n = 6) one SD of the mean were harvested following standard industry procedures. Samples of the rumen, duodenum, jejunum, ileum, colon, and ileocecal from efficiently and inefficiently fed wethers 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, Waltham, MA). Amplified DNAs were sequenced using an Illumina Miseq. Resulting sequence data was deposited in the Sequence Read Archive and is accessible through Bioproject PRJNA354123.

Sequence data were assembled with MOTHUR234 curating to remove low quality sequences using the FASTX Toolkit. Microbial diversity was measured as Shannon’s entropy with mothur’s summary-single function. Data were standardized and transformed using the vegan function vegandsvda. RPF based metrics were calculated using the nemrod function. Microbial diversity was also measured using the Anosim function, and range from 0 (identical) to 1 (no common features).

One-way ANOSIM 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 BLAST alignment to Greengenes’ nr and curated refseq, RNA databases.

Literature Cited

7. Mao et al. 2015. BMC Microbiology 15:1

Results & Conclusions

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

<table>
<thead>
<tr>
<th>Item</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM%</td>
<td>89.0</td>
</tr>
<tr>
<td>CP%</td>
<td>20.2</td>
</tr>
<tr>
<td>ADF%</td>
<td>33.2</td>
</tr>
<tr>
<td>TDN%</td>
<td>64.5</td>
</tr>
<tr>
<td>NEm, mcal/lb</td>
<td>0.65</td>
</tr>
<tr>
<td>NEg, mcal/lb</td>
<td>0.37</td>
</tr>
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</table>

**Average of samples taken from each batch (n=8) of feed used**

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

Figure 1 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

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

Figure 2 Non-metric multidimensional scaling of Bray-Curtis dissimilarity. Table 2 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 more similar.

Microbiota Are Typically More Similar To Microbiota Of Their Nearest Distal Gut Regions Than Other Gut Locations

Table 2. ANOSIM Relationships among microbiota of different GIT regions.

<table>
<thead>
<tr>
<th>GIT Region</th>
<th>Colon</th>
<th>Duodenum</th>
<th>Jejunum</th>
<th>Ileum</th>
<th>Reman</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colon</td>
<td>0.96**</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duodenum</td>
<td>0.93**</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jejunum</td>
<td>0.98**</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ileum</td>
<td>0.91**</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reman</td>
<td>1**</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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<0.05, **P<0.001

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

<table>
<thead>
<tr>
<th>Trait</th>
<th>Colony</th>
<th>Duodenum</th>
<th>Jejunum</th>
<th>Ileum</th>
<th>Remen</th>
</tr>
</thead>
<tbody>
<tr>
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<td>0.96**</td>
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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)