INSIGHTS INTO THE MICROBIOTA COMPOSITION AND METATRANSCRIPTOME AT THE GUT-BODY INTERFACE

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Microbiota of the rumen wall

- $10^{13}$ bacteria/g
- 1% of ruminal bacteria attached to rumen wall (Mueller 1984)
- Multilayered keratinized epithelium
- Form protective biofilm (McCowan 1978)
- Possibly functions: hydrolysis of urea and scavenging of oxygen (Wallace 1979), tissue recycling (McCowan et al., 1978), amino acid metabolism (Mao et al., 2015)

Fluorescence-In-Situ-Hybridization: Cy5 (blue) & green = rumen epithelium, Cy3 (red) = bacteria

Next Generation Sequencing
DNA (16S rRNA) RNA
Diversity Function

Wetzels et al., 2015
Study 1: How is the epimural ruminal microbiome constructed and does it contribute to metabolism?

Monophasic challenge model for subacute rumen acidosis (SARA)

Biphasic SARA challenge model

Sampling time points
Cows responded differently to the SARA challenge (n=8; 4 RES and 4 NRES)

<table>
<thead>
<tr>
<th>Item</th>
<th>RES</th>
<th>NRES</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline (B)</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Daily mean pH</td>
<td>6.40</td>
<td>6.44</td>
<td>0.01</td>
<td>0.82</td>
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<tr>
<td>pH below 5.8 (min/d)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>NA</td>
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<tr>
<td>Minimum pH</td>
<td>6.13</td>
<td>6.19</td>
<td>0.03</td>
<td>0.27</td>
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<tr>
<td>Maximum pH</td>
<td>6.66</td>
<td>6.65</td>
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<td>0.84</td>
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<tr>
<td>Concentrate intake (kg DM/d)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Forage intake (kg DM/d)</td>
<td>8.63</td>
<td>9.05</td>
<td>0.46</td>
<td>0.68</td>
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<tr>
<td>Adaptation (A)</td>
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<tr>
<td>Daily mean pH</td>
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<td>6.22</td>
<td>0.09</td>
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<tr>
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<td>495</td>
<td>135</td>
<td>132</td>
<td>0.21</td>
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<tr>
<td>Minimum pH</td>
<td>5.28</td>
<td>5.74</td>
<td>0.14</td>
<td>0.09</td>
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<tr>
<td>Maximum pH</td>
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<td>6.61</td>
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<tr>
<td>Forage intake (kg DM/d)</td>
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<td>6.71</td>
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<td>0.85</td>
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<tr>
<td>SARA (S)</td>
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<tr>
<td>Daily mean pH</td>
<td>5.80</td>
<td>6.38</td>
<td>0.12</td>
<td>0.01</td>
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<tr>
<td>pH below 5.8 (min/d)</td>
<td>653</td>
<td>30</td>
<td>139</td>
<td>0.03</td>
</tr>
<tr>
<td>Minimum pH</td>
<td>5.19</td>
<td>5.70</td>
<td>0.11</td>
<td>0.01</td>
</tr>
<tr>
<td>Maximum pH</td>
<td>6.34</td>
<td>6.89</td>
<td>0.12</td>
<td>0.02</td>
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<tr>
<td>Concentrate intake (kg DM/d)</td>
<td>9.10</td>
<td>10.80</td>
<td>0.74</td>
<td>0.28</td>
</tr>
<tr>
<td>Forage intake (kg DM/d)</td>
<td>5.48</td>
<td>6.66</td>
<td>0.59</td>
<td>0.36</td>
</tr>
</tbody>
</table>

1 RES were defined as cows that developed SARA (ruminal pH below 5.8 for at least 330 min/d) and NRES were defined as cows not developing SARA according to the criterion defined above.

2 Baseline was 2-wk of forage feeding, Adaptation was 1-wk adaptation to SARA diet, SARA challenge was 4-wk of SARA challenge.
Microbiota from each challenge period cluster separately
A biphasic challenge drives microbiota further distinct
Sequencing data confirmed by qPCR
Conclusions – Diversity of the epimural microbiota

- **Microbiota of the rumen epithelium are highly diverse** from the microbiota in the lumen
- *Campylobacter* and *Neisseriaceae* most abundant
- **Strong shifts** in microbiota with high-concentrate feeding
  - Independently of RES/NRES affiliation
- **Different animal response** to continuous high-concentrate diet (RES/NRES)-not explainable by diversity shifts
- **Epimural microbiota after the 2\textsuperscript{nd} SARA more distinct** to baseline than after 1\textsuperscript{st} SARA (transient feeding model)
- **Challenge break (one week) not enough** for epimural bacterial community to recover from SARA.
Metatranscriptome sequencing-based insights into the rumen wall microbiota gene expression (n=6; three each baseline and SARA challenge)

Nitrogen metabolism:
• *Flavobacterium*, *Clostridium*, *Helicobacter* (Urease)
• *Campylobacter* (dissimilatory nitrate reduction)
• *Clostridium*, *Ruminococcus*, *Fibrobacter* (Nitrogenase)

Oxydative stress response:
• *Campylobacter*, *Atopobium*, *Bifidobacterium*, *Clostridium*, *Prevotella*, *Fibrobacter*

Starch metabolism and degradation of cellulose & cellobiose:
• *Verrucomicrobia*, *Clostridium* (was thought to be a function of luminal microbiota)

Butyrate and propionate metabolism
• *Clostridium*, *Butyrivibrio*, *Burkholderia*, *Psychrobacter*, *Neisseria* (SCFA metabolism)
We found only few statistically significant differences between baseline and SARA in the metatranscriptome

- Community composition shifts; compensation at functional level?
- **Functional guilds** – different strains/species within a genus may fulfil similar functions

**EM display a vital (functional) part of the metabolism of the rumen**
(Mann et al., Front. Micro. 2018)

- Housekeeping genes were among the highest expressed genes
- **Confirmed**: Nitrogen metabolism (Urease activity), Oxidative stress response
- **New**: Starch and cellulose/cellobiose degradation
  Butyrate and propionate production
Study 2: How does AB challenge impact on fecal microbiota, attached microbiota and lymphnodes?

- 8 pigs per group (AB and control), 3 weeks adaptation phase
- 3 weeks diet, ± ABs (Colistin sulfate and Lincospectin)

<table>
<thead>
<tr>
<th></th>
<th>AB</th>
<th>Control</th>
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<tbody>
<tr>
<td></td>
<td>ICLN</td>
<td>ICLN</td>
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<tr>
<td>Ileum</td>
<td>16S rRNA gene sequencing</td>
<td>16S rRNA gene sequencing</td>
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<tr>
<td>Feces-end</td>
<td>Membrane integrity</td>
<td>Membrane integrity</td>
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<tr>
<td>Feces-start</td>
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</table>

- Study 2: How does AB challenge impact on fecal microbiota, attached microbiota and lymphnodes?
Antibiotic treatment had a significant effect on 17.4% of the OTUs in feces, 3.2% in ileal mucosa, and 1.6% in ICLN samples.

- Bacteria might escape antibiotic treatment in lymphnodes
Microbial communities separate by tissue and group

Weighted Unifrac, rarefied at 4080 sequences
Depletion of mucosa-associated segmented filamentous bacteria

$p=0.048$

From Caroline H.T. Hall, Eric L. Campbell, Sean P. Colgan: Neutrophils as Components of Mucosal Homeostasis; Cellular and Molecular Gastroenterology and Hepatology; 4, 3, 2017; Pages 329-337; Image courtesy of N.H.S. and P. Teggatz, Medical College of Wisconsin, Milwaukee, USA; (from Bevins and Salzman, 2011)
Increased methanogenesis upon antibiotic treatment?

**Sharpea**
- Gram-positive, anaerobic Firmicutes
- Associated to increased lactate formation (heterofermentative glycolysis) and low-methane emission (competes for H2)

**Methanobrevibacter**
- Methanogenic Archaea
- Quickly occupying freed niches from bacteria that have been killed by the ABs
- Likely contributing to increased carbohydrate metabolism
Conclusions

- As for gut microbiota, microbiota of ileal mucosa and ileocaecal lymphnodes (ICLN) represent unique corresponding environmental microbial niches.

- AB challenge had a remarkable impact on the gut (fecal) microbiome, but less impact on ileum-attached OTUs and almost none on the lymphnode microbiome. Protective microbial mechanisms involved or only a pharmacokinetic effect?

- Evidence that Proteobacteria (e.g. EPEC) could escape antibiotic treatment, if they are translocated to lymph nodes (risk factor during slaughtering and meat cutting (incision)).

- AB treatment of livestock might have effects on global biogeochemical cycles.
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