Discover potential regulatory mechanisms involved in rumen functional changes under high grain diet

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EAAP 69\textsuperscript{th} Annual Meeting, Dubrovnik, Croatia
Functional Genomics and Microbiology at University of Alberta

Molecular profiling of microbial community
- Metagenomics, metatranscriptomics of gut microbiome
- Host transcriptome and microRNA profiling
- Metabolomics

- Rumen
  - Feed efficiency
  - Methane emission
  - Rumen Acidosis

- Gut
  - Host innate Immunity
  - Barrier function

https://www.cattleomics.com/
SCFA absorption accounted for up to 53% of the ruminal buffering capacity.
Distinct individual variation – adapt to HGD

High grain diet (HGD)

Higher ruminal pH

Lower ruminal pH

(Bevans et al., 2005; Mahammed et al., 2012; Penner et al., 2009)
Hypothesis and objectives

• Hypothesis
  – The HGD change gene expression at whole transcriptome level
  – Individual variation during HGD adaptation can be explained by transcriptome variation of ruminal epithelium

• Objectives
  – To characterize transcriptome of ruminal epithelia during HGD transition using RNA-seq
  – To compare ruminal epithelia transcriptomes focusing on the difference of individual adaptation
Experimental design

Rumen papillae and RNA extraction (n = 45)

Transcriptome profiles

<table>
<thead>
<tr>
<th>d3 (n = 15)</th>
<th>d15 (n = 15)</th>
<th>d27 (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3% grain</td>
<td>40% grain</td>
<td>85% grain</td>
</tr>
<tr>
<td>(d 1-4)</td>
<td>(d 5-8)</td>
<td>(d 17-20)</td>
</tr>
<tr>
<td>60% grain</td>
<td>75% grain</td>
<td>92% grain</td>
</tr>
<tr>
<td>(d 9-12)</td>
<td>(d 13-16)</td>
<td>(d 21-28)</td>
</tr>
<tr>
<td>75% grain</td>
<td>85% grain</td>
<td></td>
</tr>
<tr>
<td>(d 13-16)</td>
<td>(d 17-20)</td>
<td></td>
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<tr>
<td>85% grain</td>
<td></td>
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<td>92% grain</td>
<td></td>
<td></td>
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<tr>
<td>(d 21-28)</td>
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</tr>
</tbody>
</table>

RNA sequencing

Illumina HiSeq 2000

Differentially expressed (DE) genes

Tophat2, HT-seq

Differentially expressed (DE) genes

EdgeR

Functional analysis

Potential regulatory mechanisms of individual variation during HGD adaptation
Transcriptome profiling under different grain diets

PC1 13.77%

PC2 12.15%

-100 -50 0 50 100

-100 -50 0 50 100 150

PC1 (13.77%)
PC2 (12.15%)

3% grain
75% grain
92% grain
The genes with reads per million (RPM) > 1 in 15 out of 15 cattle in at least 1 diet
Different pH trend during dietary adaptation
Acidosis index showed similar result with ruminal pH.
More DE genes were found in UG heifers

DG (92% vs. 75%)

- 67 genes
  - 22-up regulated
  - 45-down regulated

UG (92% vs. 75%)

- 285 genes
  - 122-up regulated
  - 163-down regulated

(RPM >1 in 5 out of 5 cattle in at least 1 diet, FC >1.5 or < -1.5 and FDR <0.05)
Intracellular pH was differentially regulated in DG and UG

<table>
<thead>
<tr>
<th></th>
<th>DG-75%</th>
<th>DG-92%</th>
<th>UG-75%</th>
<th>UG-92%</th>
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</thead>
<tbody>
<tr>
<td>NHE1</td>
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<tr>
<td>NHE2</td>
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<tr>
<td>NHE3</td>
<td></td>
<td></td>
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<tr>
<td>NHE6</td>
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<td>NHE8</td>
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<tr>
<td>NHE9</td>
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</tr>
<tr>
<td>MCT1</td>
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<tr>
<td>ATP1B1</td>
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<tr>
<td>ATP1B3</td>
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</tbody>
</table>

92% vs. 75%

- **DG**
- **UG**

- **Na⁺/H⁺ exchanger**
- **Na⁺/K⁺ exchanger**
- **Anion exchanger**

(T-test, * p < 0.1, ** p < 0.05, and *** p < 0.001)
Lipid metabolism was differently regulated in DG and UG

Top two functions of Up-regulated genes in DG

<table>
<thead>
<tr>
<th>Diseases or Functions Annotation</th>
<th>P-Value</th>
<th>Z-score</th>
<th>Molecules</th>
</tr>
</thead>
<tbody>
<tr>
<td>Up-regulated genes (n = 22)</td>
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<tr>
<td>Concentration of lipid</td>
<td>9.29E-03</td>
<td>1.94</td>
<td>6</td>
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<tr>
<td>Concentration of triacylglycerol</td>
<td>9.29E-03</td>
<td>1.70</td>
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</table>

Top two functions of Up-regulated genes in UG

<table>
<thead>
<tr>
<th>Diseases or Functions Annotation</th>
<th>P-Value</th>
<th>Z-score</th>
<th>Molecules</th>
</tr>
</thead>
<tbody>
<tr>
<td>Up-regulated genes (n = 122)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Storage of lipid</td>
<td>3.85E-03</td>
<td>-2.00</td>
<td>5</td>
</tr>
<tr>
<td>Oxidation of lipid</td>
<td>1.09E-02</td>
<td>2.00</td>
<td>7</td>
</tr>
</tbody>
</table>
Expression of FABP4 increased in DG, FABP5 decreased in UG

FABPs are lipid chaperone protein, which associated with lipogenesis and fat deposition

Long chain fatty acid transporter

Cholesterol transporter

(T-test, * p < 0.1, ** p < 0.05, and *** p < 0.001)

(Specht et al., 1996; Hertzel et al., 2006; Michal et al., 2006)
Expression of ketogenesis and cholesterol synthesis related genes was increased in DG but deceased in UG

(T-test, * p < 0.1, ** p < 0.05, and *** p < 0.001)
Summary

High grain diet (72% to 89% grain) → Cellular damage

Down group:
- Innate immunity ↓
- Inhibited defense
- Cholesterol ↑
- Cholesterol accumulate
- Cellular stability/homeostasis
- Normal function of epithelia
- Lumen pH ↓

Up group:
- Cell cycle arrest ↑
- Repair
- Xenobiotic metabolism ↑
- Elimination of toxins
- Cellular stability/homeostasis
- Normal function of epithelia
- pH ↑
Summary

- Dietary grain concentration affected gene expression of ruminal epithelium at whole transcriptome level
- Transcriptional regulation of lipid transport, fatty acid metabolism, and intracellular homeostasis might be the molecular mechanism accounting for individual variation during the diet transition to a high grain diet
- The identified genes could be potential gene markers for selecting cattle with maintained ruminal pH through a diet transition to a high grain diet
Transcriptome analysis of ruminal epithelia revealed potential regulatory mechanisms involved in host adaptation to gradual high fermentable dietary transition in beef cattle

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Acknowledgement

All the co-authors

Dr. K. Zhao (Shanxi Normal University)
Dr. M. Oba (University of Alberta)
Dr. G. Penner (University of Saskatchewan)

All the members in my lab