Influence of feed efficiency and physiological state on rumen VFA and microbial profiles in cattle

S Lam¹, J Munro², J Cant¹, L Guan³, M Steele³, F Schenkel¹, S Miller¹,4, Y Montanholi²
Outline

• Introduction
• Hypothesis
• Objectives
• Materials and methods
  – Grain-fed study
  – Grass-fed study
• Results and discussion
• Conclusion
• Acknowledgements
Introduction: Industry challenges

30-50% agriculture GHG
Introduction: Industry challenges

30-50% agriculture GHG

Feed costs

50% Herd cost variation
Introduction: Feed efficiency
Introduction: Feed efficiency
Introduction: Feed efficiency

- Milk and colostrum composition 
  (Montanholi et al. 2013)
- Blood metabolites and hormones 
  (Kelly et al. 2011, Gonano et al. 2014)
- Fertility traits 
  (Awda et al. 2013, Fontoura et al. 2015)
- Cardiac physiology 
  (Munro et al. 2016)
Introduction: Rumen metabolism

10% of the biological variation of RFI due to digestibility
(Richardson and Herd 2004)

Rumen and reticulum = 75% total digestive tract
(Baldwin 1980)

Large energetic sink with high energy demand
(Hungate 1960)

Area of energy absorption - 75% total VFA
(Bergman 1990)
Introduction: Microbiology

Bacteria ($10^{11}$ cells/ml)
75% feed particle digestion

Fungi ($10^3-10^4$ cells/ml)
Fibrolytic particle digestion

Methanogens ($10^4-10^6$ cells/ml)
Methanogen ecology associated with methane emissions

Protozoa ($10^4-10^6$ cells/ml)
Ciliate species digesting suspended and colonized feed particles

Introduction: Volatile fatty acids (VFA)

- Feed efficiency
- 75% energy requirements

(Guan et al. 2008, Hernandez-Sanabria 2012)

(Briggs et al. 1957, Bergman et al. 1990)
Introduction: Rumen microstructure

Sheep papillae microstructure

Low energy diet:

High energy diet:

Stratum corneum thickness

Energy in diet

(Steele et al. 2012)
Introduction: Rumen pH

\[ pH = 0 \]

\[ pH = 7 \text{ neutral} \]

\[ pH = 14 \text{ alkaline} \]

\[ pH = 14 \]

\[ pH = 7 \]

\[ pH = 0 \]

(Kimura et al. 2016)
Feed efficiency is associated with energetic processes and the rumen is a highly metabolically active organ. Therefore, the variability in rumen metabolism across feed efficiency phenotypes and dietary treatments may be featured through rumen functional and structural assessments.
Objectives

Microbiology

Papillae epithelium

Volatile fatty acids (VFA)

Rumen pH

Objectives
Objectives

Efficient vs Inefficient

Grain-fed animals

Grass-fed animals

Microbiology

Papillae epithelium

Volatile fatty acids (VFA)

Efficient vs Inefficient

Grain-fed animals

Grass-fed animals

Rumen pH
Feedlot study

- 48 crossbred cattle
- Trial length: 112 d

Average BW = 536 ± 43 kg

Average BW = 506 ± 72 kg

Average BW = 531 ± 57 kg

Overall breed composition

Angus: 44.9%
Simmental: 39.4%
Other breeds: 15.7%

Elora Beef Research Centre
Grass-fed study

- 141 crossbred cattle
- Trial length: 124 d

Materials: Experimental conditions

Overall breed composition

- Angus: 55.2%
- Simmental: 23.6%
- Other breeds: 21.2%

107 heifer calves

- BW = 253 ± 38 kg
- Age = 403 ± 27 d

36 pregnant heifers

- BW = 406 ± 42 kg
- Age = 594 ± 95 d
Methods: Diet

*Contains 40% of calcium phosphate, 60% trace mineralized salt

<table>
<thead>
<tr>
<th>Chemical Composition</th>
<th>Dry Basis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry Matter</td>
<td>53.8</td>
</tr>
<tr>
<td>Crude Protein</td>
<td>13.9</td>
</tr>
<tr>
<td>Acid Detergent Fibre</td>
<td>10.9</td>
</tr>
<tr>
<td>Neutral Detergent Fibre</td>
<td>22.2</td>
</tr>
<tr>
<td>Starch</td>
<td>45.0</td>
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<tr>
<td>Total Digestible Nutrients</td>
<td>86.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ingredient Composition</th>
<th>Dry Basis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High-moisture corn</td>
<td>52.5</td>
</tr>
<tr>
<td>Haylage</td>
<td>42.3</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>3.5</td>
</tr>
<tr>
<td>Premix*</td>
<td>1.7</td>
</tr>
</tbody>
</table>

* Contains 40% of calcium phosphate, 60% trace mineralized salt.
Methods: Diet

**GROWSAFE feeding system**

*Contains 37.4% of calcium phosphate, 62.7% trace mineralized salt

### Chemical Composition

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<th>Ingredient</th>
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<tr>
<td>Dry matter</td>
<td>36.1</td>
</tr>
<tr>
<td>Crude Protein</td>
<td>15.5</td>
</tr>
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<td>Acid Detergent Fibre</td>
<td>29.6</td>
</tr>
<tr>
<td>Neutral Detergent Fibre</td>
<td>53.7</td>
</tr>
<tr>
<td>Starch</td>
<td>6.5</td>
</tr>
<tr>
<td>Total Digestible Nutrients</td>
<td>70.3</td>
</tr>
</tbody>
</table>

### Ingredient Composition

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Dry Basis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haylage</td>
<td>99.5</td>
</tr>
<tr>
<td>Premix*</td>
<td>0.5</td>
</tr>
</tbody>
</table>

*Contains 37.4% of calcium phosphate, 62.7% trace mineralized salt
Methods: RFI models

Grain-fed ($R^2 = 0.74$):

Feed intake = $[\mu + (\beta_1 \times \text{body weight})] + (\beta_2 \times \text{ADG}) - (\beta_3 \times \text{ribeye area}) + (\beta_4 \times \text{back fat}) - (\beta_5 \times \text{marbling}) + \text{subpopulation} + \text{RFI}$

Grass-fed ($R^2 = 0.61$):

Feed intake = $[\mu + (\beta_1 \times \text{body weight})] + (\beta_2 \times \text{ADG}) - (\beta_3 \times \text{ribeye area}) + (\beta_4 \times \text{rump fat}) - (\beta_5 \times \text{age}) + \text{subpopulation} + \text{RFI}$
Methods: Sample collection

5.5±1 d prior slaughter

End of performance test

pH logger insertion

Rumen fluid

Rumen tissue
Methods: Logger insertion

Rumen pH loggers
T9 LRCpH Data Logger Dascor

**Method:** esophageal tubing

**Recording:** 5 minute intervals
(~2,600 data points/animal)
Methods: Rumen fluid collection

Method:
Oro-ruminal probe with suction

Evaluating:
Microbiology
Volatile fatty acid profiles
Methods: Microbiology

Method:
Rumen fluid DNA isolation
RT-qPCR

Evaluating:

- Bacteria
- Protozoa
- Fungi
- Methanogen

Ruminal fluid DNA analysis:

(Doddema 1978; Mathers and Miller 1980; Godfried 1980)
Methods: VFA

**Evaluated:**

- VFA molar concentrations
  - Acetate
  - Propionate
  - Butyrate
  - Valerate
  - Isovalerate
  - Isobutyrate
  - Caproate

**Total VFA concentration**

**Method:**

- VFA sample processing

Bruker, CP-8400 Autosampler
Methods: Rumen tissue collection

Method:
Tissue collection
Processed for histomorphology

Evaluating: Papillae epithelial thickness
Methods: Histomorphometry

Histology traits:
- Stratum corneum
- Papillae width
Methods: pH measurements

Rumen pH vs Time (hh:mm)

- High-RFI Predicted pH
- High-RFI Observed pH

Methods: pH measurements
Univariate Normality Procedure
- Skewness, Kurtosis, Anderson-Darling Test
- Transformations
  - logarithm
  - squared

GLM Select procedure
- Determine model effects

General Linear Model (GLM) Procedure
- Rumen traits

Partial Least Square procedure
- Determine % contribution to RFI

\[ Y_{ijkl} = \mu + \text{efficiency group}_i + \text{subpopulation}_j + \text{breeds}_k + \epsilon_{ijkl} \]
## Results: Physiological status

<table>
<thead>
<tr>
<th>Trait (%/total VFA)</th>
<th>Heifer calves</th>
<th>Pregnant heifers</th>
<th>( P)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetate</td>
<td>71.68</td>
<td>74.62</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Propionate</td>
<td>18.30</td>
<td>16.53</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Isobutyrate</td>
<td>0.97</td>
<td>0.89</td>
<td>0.051</td>
</tr>
<tr>
<td>Butyrate</td>
<td>7.29</td>
<td>6.47</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Isovalerate</td>
<td>0.90</td>
<td>0.90</td>
<td>0.952</td>
</tr>
<tr>
<td>Valerate</td>
<td>0.58</td>
<td>0.41</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Caproate</td>
<td>0.28</td>
<td>0.18</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Total VFA ((\mu)mol/ml)</td>
<td>43.52</td>
<td>37.39</td>
<td>&lt;0.01</td>
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Pregnant heifers

Bacteria population

Metabolic activity throughout gestation

VFA metabolism and energy demand

(Church, 1988; Drackley et al. 2001)
## Results: RFI – Microbial profiles

<table>
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<tr>
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<th>Inefficient</th>
<th>Efficient</th>
<th>P-value</th>
</tr>
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<tbody>
<tr>
<td>Total bacteria</td>
<td>4.3x10^{11}</td>
<td>7.6x10^{11}</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Methanogen</td>
<td>4.9x10^{9}</td>
<td>2.3x10^{9}</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Protozoa</td>
<td>4.3x10^{7}</td>
<td>1.5x10^{7}</td>
<td>0.18</td>
</tr>
<tr>
<td>Fungi</td>
<td>6.3x10^{4}</td>
<td>3.8x10^{4}</td>
<td>0.37</td>
</tr>
</tbody>
</table>

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<tr>
<td>Total bacteria</td>
<td>6.0x10^{10}</td>
<td>5.3x10^{10}</td>
<td>0.16</td>
</tr>
<tr>
<td>Methanogen</td>
<td>2.6x10^{7}</td>
<td>3.1x10^{7}</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Protozoa</td>
<td>1.2x10^{5}</td>
<td>1.6x10^{5}</td>
<td>0.30</td>
</tr>
<tr>
<td>Fungi</td>
<td>1.3x10^{5}</td>
<td>1.9x10^{5}</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Trait (%/total VFA)</td>
<td>Inefficient</td>
<td>Efficient</td>
<td>P-value</td>
</tr>
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<td>---------------------</td>
<td>-------------</td>
<td>-----------</td>
<td>---------</td>
</tr>
<tr>
<td>Acetate</td>
<td>54.4</td>
<td>54.5</td>
<td>0.97</td>
</tr>
<tr>
<td>Propionate</td>
<td>29.0</td>
<td>29.3</td>
<td>0.89</td>
</tr>
<tr>
<td>Isobutyrate</td>
<td>1.2</td>
<td>1.0</td>
<td>0.11</td>
</tr>
<tr>
<td>Butyrate</td>
<td>8.9</td>
<td>8.6</td>
<td>0.75</td>
</tr>
<tr>
<td>Isovalerate</td>
<td>3.0</td>
<td>2.7</td>
<td>0.41</td>
</tr>
<tr>
<td>Valerate</td>
<td>2.6</td>
<td>2.9</td>
<td>0.29</td>
</tr>
<tr>
<td>Caproate</td>
<td>0.4</td>
<td>0.5</td>
<td>0.40</td>
</tr>
<tr>
<td>Total VFA (µmol/ml)</td>
<td>78.0</td>
<td>80.3</td>
<td>0.66</td>
</tr>
</tbody>
</table>
Results: Variance analysis

32.5%  21.5%
Results: Variance analysis

- Bacteria: 32.5%
- Methanogen: 44.3%
- Protozoa: 24.1%
- Fungi: 25.8%

- Bacteria: 25.1%
- Methanogen: 25.1%
- Protozoa: 24.9%
- Fungi: 24.9%

- Bacteria: 10.0%
- Methanogen: 21.5%
Results: Variance analysis

- Bacteria: 25.5%
- Methanogen: 24.1%
- Protozoa: 25.8%
- Fungi: 24.9%

- Acetate: 19.0%
- Propionate: 36.2%
- Isobutyrate: 9.8%
- Butyrate: 19.9%
- Isovalerate: 8.6%
- Valerate: 5.5%
- Caproate: 0.9%

- Other: 55.7%
Results: Papillae histomorphometry

<table>
<thead>
<tr>
<th>Papillae width</th>
<th>Papillae area</th>
<th>Inefficient</th>
<th>Efficient</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>(µm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Base</td>
<td>110.1</td>
<td>113.4</td>
<td>0.39</td>
<td></td>
</tr>
<tr>
<td>Middle</td>
<td>119.5</td>
<td>139.4</td>
<td>&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>Tip</td>
<td>125.0</td>
<td>148.4</td>
<td>&lt;0.01</td>
<td></td>
</tr>
</tbody>
</table>
Results: Predicted rumen pH curves

![Graph showing predicted rumen pH curves with time of day (hh:mm) on the x-axis and rumen pH on the y-axis. The graph compares two conditions: Inefficient and Efficient. A separate line represents feed intake.](image-url)
Results: Predicted rumen pH curves

* = P < 0.05
Results: Predicted rumen pH curves

- 5.6 < pH < 6.0
- Inefficient 4.4% vs Efficient 8.5%
- P = 0.02

* = P < 0.05
Summary

Feed Efficiency

Bacteria population
Summary

Feed Efficiency

- Bacteria population
- Methanogen population

Methanogen population
Summary

Feed Efficiency

- Bacteria population
- Methanogen population
- Total and specific VFA
Summary

Feed Efficiency

- Bacteria population
- Methanogen population
- Papillae width
- Circadian pH
- Methanogen population
- Total and specific VFA
Conclusions

• Rumen microbiology, functional and structural parameters are important in assessing the underlying digestive biology of feed efficiency.

• Dietary treatment has an impact on the relevance of rumen parameters used for indicating feed efficiency.
Acknowledgements

- Advisor: Dr. Yuri Montanholi
- Colleagues and volunteers
- Technical staff
  Yanhong Chen
  Tim Caldwell
- Elora beef research centre
- Maritime beef testing station