Rumen Function and Metabolism

Basic Dairy Nutrition Course
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Today’s topics:

- Rumen environment
- Rumen fermentation
- Fermentation and acid load
- Rumen dynamics and kinetics
- HOP TOPIC – methanogenesis
The rumen environment

- A large fermentation vat
- This environment is maintained by the “host” and supports a symbiotic relationship with rumen microorganisms
- How does the rumen work?
  - [https://www.youtube.com/watch?v=muf8ZA3F2mA](https://www.youtube.com/watch?v=muf8ZA3F2mA)

Structures of the rumen
A mutualistic symbiosis

- The ruminant host provides:
  - Large fermentation vat
  - Low oxygen environment
    - Microbes are anaerobic
  - Nutrient supply
  - Water
  - Constant temp (99 - 106°F)
  - Constant mixing
  - Buffered pH (pH 7.0 to 5.5)
  - Rumination to decrease particle size
  - Removal of end products

- The microbes provide:
  - β-linked CHO cleavage enzymes
  - VFA for energy and milk synthesis
  - Non-protein nitrogen → amino acids
  - Good quality protein
  - Vitamin synthesis (B-vitamins and vitamin K)
  - Essential FA synthesis
  - Detoxification of mycotoxins

Who are we feeding? The cow or her rumen microbes?

A mutualistic symbiosis

• Advantages (this gives ruminants an important niche):
  – Nutrient recycling
  – Nutrient synthesis
  – Byproduct feeding

• Disadvantages:
  – Proteins (of very high quality) are also degraded; re-made into microbial proteins of good, but lesser quality
  – Loss of energy from diet gross energy as heat of fermentation (4 to 6%) and methane gas (3 to 12%)
  – More on methanogenesis later...

What are some evolutionary advantages?

Ku-Vera et al., 2020

Credit for Use of Human Food & Fiber By-products Further Reduces the Carbon-Footprint of Milk

Oil extraction
  • Almond hulls
  • Canola meal
  • Cotton hulls
  • Cotton seed meal
  • Linseed meal
  • Peanut meal
  • Soy hulls & meal
  • Sunflower hulls & meal

Brewing & Spirits
  • Brewers grains
  • Brewers solubles
  • Brewers yeast
  • Distillers grains

Grain milling
  • Bran (corn, wheat, rice)
  • Cereal fines
  • Midds (corn, wheat, barley)

Clothing
  • Whole cotton seed

Fruit/vegetable processing
  • Pomace (apple, tomato, carrot)
  • Vine silage (peas & legumes)
  • Corn stover
  • Potato peels

Citrus processing
  • Citrus pulp

Ethanol production
  • Distillers grains (corn, milo, barley, sorghum)

Dry corn milling for corn flour & grits
  • Corn bran
  • Hominy feed

Wet corn milling for starch, sweeteners & oil
  • Corn germ meal
  • Corn gluten feed

Sugar processing
  • Beet pulp
  • Molasses

Fish processing
  • Fish meal

Cheese manufacturing
  • Whey

Baking industry
  • Bakery by-products
  • Expired product

Chocolate manufacturing
  • Candy by-products
  • Confectionary waste

Human foods that fail grading
  • Starches, oils
  • Grains, flour
  • Vegetables

Courtesy of T.R. Overton
On to fermentation!

**fer-men-ta-tion**

-the chemical breakdown of a substance by bacteria, yeasts, or other microorganisms

-the extraction of energy from carbohydrates in the absence of oxygen

*Thanks Wikipedia!*
Major players?

The Secret Life of Microbes, By Paul Weimer

Rumen microbes

- Rumen volume in a lactating cow ≈ 120 L

- Approx. 13 billion microbes per mL of rumen fluid
  - Over 200 species
  - We’ve only cultured and identified ~10%
  - Advancements in molecular biology techniques have allowed us to describe more features of the rumen microbiome

- Rumen microbes:
  - Bacteria, 93.5% (ferment fiber, starch, sugars, protein, and more)
  - Protozoa, 2% (eat bacteria, ferment starch)
  - Archaea, 2% (produce methane gas)
  - Fungi, 2.5% (help break down fiber)

McSweeney et al., 2005; Romagnoli et al., 2017.
Rumen microbiome

Henderson et al., 2015. Rumen microbial community composition varies with diet and host, but a core microbiome is found across a wide geographical range. Scientific Reports. 5:14567.

“The rumen contains one of the most diverse and dense microbial ecologies known in nature.” – Stewart et al., 2019. Nature Biotech.

Core rumen microbiome

Rumen microbes are often classified by their substrate preference or the end products they produce.

What is getting fermented?

Van Soest Detergent System for Fiber Analysis

- Plant Carbohydrates
  - Cell Contents
    - Organic acid
    - starch
    - fructans
    - sugar
  - Cell Wall
    - Pectin
    - B-glucans
    - galactans
    - hemicellulose
    - cellulose
    - lignin

- Neutral detergent
  - Soluble Fiber
    - Soluble CHO
    - Nonstructural CHO

- Neutral Detergent Solubles

CHO = carbohydrates

Adapted from Ishler and Varga, DAS03-29, Penn State Extension

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CHO fractions and Digestibility

- Carbohydrates
  - Soluble Carbohydrates
  - Fiber Cell Walls
    - NDF*
    - ADF**

- Sugars
- Starch
- Pectin
- Hemicellulose
- Cellulose
- Lignin

Digestibility %:
- 95-100% Very good digestibility
- 20-80% Medium to good digestibility
- 0-20% Poor digestibility

Adapted from Ishler and Varga, DAS03-29, Penn State Extension
CHO fermentation

• Major energy-yielding substrates: Cellulose, hemicellulose, pectin, starch, fructan (these are polysaccharides: polymers of sugars)
• CHO are main source of dietary energy because they are fermented to volatile fatty acids (VFA) for use by the animal
• Fibrous CHO (NDF) (structural CHO)
  – Cellulose and hemicellulose
  – Medium to good digestibility
• Non-fibrous carbohydrates (NFC) (non-structural CHO)
  – Primarily starch
  – Very good digestibility

Rumen fermentation pathways (*simplified*)

Dietary polysaccharides: cellulose, hemicellulose, pectin, starch

\[
\begin{align*}
\text{Hydrolysis} \\
\text{Soluble sugars (simple sugars): glucose, fructose} \\
\text{Hydrolysis and fermentation} \\
\text{Intermediary metabolic compounds (pyruvate, lactate, formate, succinate)}
\end{align*}
\]

\[
\begin{align*}
\text{Fermentation} \\
\text{Acetate} \\ 
\text{Butyrate} \\ 
\text{Propionate} \\
\text{VFA = Energy sources for the host}
\end{align*}
\]

\[
\begin{align*}
\text{Methanogenesis} \\
\text{CH}_4 + \text{CO}_2 \\
\text{Eructed gas}
\end{align*}
\]

\[
\begin{align*}
\text{H}_2 + \text{CO}_2
\end{align*}
\]

\[
\text{VFA} = \text{Energy sources for the host}
\]

\[
\text{Eructed gas}
\]
Fiber and Starch Digestion

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Fiber Digesting Bacteria</th>
<th>NSC Digesting Bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preferred energy source</td>
<td>Available cell wall</td>
<td>Starch and Sugar</td>
</tr>
<tr>
<td>Preferred N source</td>
<td>Ammonia N</td>
<td>Ammonia and Peptides</td>
</tr>
<tr>
<td>pH sensitivity</td>
<td>More pH sensitive (~6.2 – 6.8 optimum)</td>
<td>Less pH sensitive (~5.2 – 6.0 optimum)</td>
</tr>
<tr>
<td>VFA produced</td>
<td>Acetate, Butyrate</td>
<td>Propionate (lactic?)</td>
</tr>
<tr>
<td>Growth rate</td>
<td>Slower</td>
<td>Faster</td>
</tr>
</tbody>
</table>

Fermentation

- Major energy-yielding substrates: Cellulose, hemicellulose, pectin, starch, fructan, organic acids, and protein

- Fermentation products:
  - Gases: CO$_2$ and methane (CH$_4$)
  - Water and Heat
  - Volatile fatty acids (VFA)
    - Acetate, propionate, butyrate
  - Microbial growth!
Microbial protein

- Dietary protein (rumen degradable protein) is broken down in the rumen to ammonia N
  - Rumen microbes incorporate ammonia N into microbial protein
    - Continual microbial turnover
  - Microbes pass out of the rumen and are a source of metabolizable protein for the animal
- Other sources of protein for the cow?
  - Rumen undegradable protein (RUP)
  - Endogenous protein from sloughed rumen and intestinal cells

VFA or Short Chain Fatty acids (SCFA)

- These are waste products to the microbes, but the major energy source for the cow
- Contribute 75% of total metabolizable energy per day

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Heat of combustion, kcal/mole</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>670</td>
</tr>
<tr>
<td><strong>Products</strong></td>
<td></td>
</tr>
<tr>
<td>Acetate</td>
<td>209</td>
</tr>
<tr>
<td>Propionate</td>
<td>365</td>
</tr>
<tr>
<td>Butyrate</td>
<td>522</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>VFA</th>
<th>ATP mol/mol of substrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetate</td>
<td>2</td>
</tr>
<tr>
<td>Propionate</td>
<td>2</td>
</tr>
<tr>
<td>Butyrate</td>
<td>1.5</td>
</tr>
</tbody>
</table>

How much ATP from aerobic metabolism (oxidative phosphorylation) of glucose??
Fates of absorbed VFA

• Acetate (mostly from fiber digestion)
  – 2-carbon VFA
  – Main precursor for milk fat synthesis
  – Adipose tissue and mammary gland use acetate to make long chain fatty acids

• Butyrate (mostly from starch digestion)
  – 4-carbon VFA
  – Mostly metabolized by the rumen wall as an energy source

• Propionate (mostly from starch digestion)
  – 3-carbon VFA
  – GLUTONEOGENESIS in the liver
  – Energy supply to the cow and to make milk lactose

Fermentation and Acid Load

For a cow producing ~105 lb milk and eating 60 lb DM:
120 moles of fermentation acids can be produced
Percent of VFA might be 55% Acetate, 30% Propionate, 15% Butyrate

How much acid is this?

<table>
<thead>
<tr>
<th>VFA</th>
<th>Total moles</th>
<th>Proportion of VFA</th>
<th>MW, g/mol</th>
<th>Total g of VFA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetate</td>
<td>120</td>
<td>0.55</td>
<td>60.05</td>
<td>3,963</td>
</tr>
<tr>
<td>Propionate</td>
<td>120</td>
<td>0.30</td>
<td>74.08</td>
<td>2,667</td>
</tr>
<tr>
<td>Butyrate</td>
<td>120</td>
<td>0.15</td>
<td>88.11</td>
<td>1,586</td>
</tr>
</tbody>
</table>

That is over 8 kg or almost 18 lb of acid every day!
**Rumen pH and acidosis**

**Figure 1.** Relationship between ruminal pH and the associated changes in the ruminal environment and ruminal epithelial function.

*Penner & Aschenbach, 2014. Mechanisms of Acid Absorption in the Rumen and Impacts on Subacute Rumen Acidosis (SARA).*

**Dealing with Acid Load: VFA absorption**

Dealing with Acid Load: Buffering

**Buffering the Rumen**
- 30% from saliva
  - 20 to 50 gal of saliva per day
- 40% from the rumen wall
  - In exchange for VFA
- 5% from fluid passage
- 25% is ???

**Common dietary buffers**
- Sodium bicarbonate
- Sodium sesquicarbonate
- Magnesium oxide
- Potassium carbonate
  - Can make up 1% of diet DM

Why do we care about rumen function and metabolism?

- By optimizing
  - Rumen function
  - Microbial growth
  - VFA production

- We can maximize
  - Milk and component yields
  - Feed efficiency
  - Rumen health
  - Cow health
Rumen dynamics

- Interactions between substrates and microorganisms and inter-microbial interactions:
  - Hydrolysis
  - Fermentation
  - Biohydrogenation
  - Microbial growth and synthesis

- Includes the host and diet factors that influence these interactions:
  - Intake
  - Rumination and eructation
  - Absorption and buffering
  - Components and physical characteristics of the diet

A quick pass with low digestibility on rumen kinetics

- When a feed particle enters the rumen, it can only leave by one of two mechanisms
  - Fermentative digestion (60 to 85% of organic matter)
  - Passage into the omasum

- These two processes compete with each other

- We term them:
  - $kd$: fractional rate of degradation
    - Rumen degradation, $\% = \left[ \frac{kd}{(kp + kd)} \right] \times 100$
  - $kp$: fractional rate of passage
    - Rumen escape, $\% = \left[ \frac{kp}{(kp + kd)} \right] \times 100$
How do we use $kd$ and $kp$?

- Microbial digestion of fiber is a slow process
- The slower feed particles pass from the rumen, the more exposure to microbes and thus more digestion
- **BUT**
- When feed particles remain in the rumen for digestion, then it limits gut space available for new feed and intake is limited
- Passage rate ($kp$) and degradation rate ($kd$) are essential to understanding the extent of digestion

Why do we talk about rumen dynamics?

- Our understanding of rumen dynamics allows us to manipulate them
- DMI, diet composition and digestibility control production responses and influence health
- Ration formulation software is built on rumen dynamics
- Almost all conventionally managed cows are fed using models that are based on rumen kinetics and dynamics
  - E.g. CNCPS biology feeds 50% of cows in the U.S. ~5 million cows and >10 million total cows worldwide
- Key factors we measure or predict:
  - Feed intake, gut fill, passage and degradation rate
**HOT TOPIC - methane (CH₄)**

- What is the role of enteric CH₄ production in the rumen?

- Methanogenesis is the main biochemical pathway for the removal of metabolic hydrogen (H₂) released from fermentation of carbohydrates in the rumen.

- The chemical reaction for CH₄ synthesis is:
  \[ \text{CO}_2 + 4\text{H}_2 \rightarrow \text{CH}_4 + 2\text{H}_2\text{O} \]

- Converting CHO to fermentable sugars relies on the effective disposal of H₂ through reduction of CO₂ to methane by methanogens.

- **BUT...**

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**HOT TOPIC - methane (CH₄)**

- CH₄ production represents an energy loss of 3 to 12% of gross energy.

- The type of carbohydrate being fermented and the composition of the rumen microbiome determine CH₄ synthesis.

- There is potential and benefits to manipulating the fate of metabolic hydrogen away from CH₄ toward propionic acid formation.

- One way to do this is...
*Monensin (ionophore antibiotic)*

- Ionophores are hydrophobic molecules produced by *Streptomyces* species and dissolve in membranes and destroy ion gradients.

- Cattle fed monensin have greater feed efficiency and produce less methane

- In the rumen, monensin
  - Selectively prohibits bacteria that produce H₂ and reduces H₂ availability for methanogenesis (reduces carbon loss and improves energy efficiency)
  - Decreases the proportions of acetate and butyrate and increases propionate production (improves energy efficiency)
  - Reduces ruminal degradation of protein (improves protein utilization)

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Ideal rumen fermentation

- Rapid rates of fiber digestion
- Rapid and efficient microbial growth
- Little ammonia accumulation
- Little methane production
- Optimal ratios of VFA
- Little lactate
- Fewer toxins

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Goals for feeding dairy cattle

• Balanced TMR and excellent feeding management
• Maximize DMI
• Optimize nutrient digestibility
• Optimize rumen function

• Why?
  – Productivity
  – Profitability
  – Animal health and welfare