Nitrogen Metabolism in Ruminants

Feed the bugs…
Feed the cow…..

Digestion in the Rumen:
Anaerobic Fermentation
It's All About the Bugs!

- Rumen volume lactating cow = 120,000 ml
- Rumen bacteria: 10,000,000,000/ml
- Rumen protozoa: 1,000,000/ml
- Rumen fungi: 1,000/ml

Ruminant vs Nonruminant –
Similarities in N Metabolism
1. At tissue level – Metabolic pathways similar
2. Can synthesize dispensable AA
3. Cannot synthesize indispensable AA
   - Essential AA must be provided from digestive tract
4. Tissue proteins constantly undergoing turnover
5. AA not stored
6. Constant supply of AA required

Ruminant vs Nonruminant –
Dissimilarities in N Metabolism
1. Microbial population has profound effect on AA reaching S.I.
   a. AA profile at S.I. different from diet
      - Up-grades low quality dietary protein
      - Down-grades high quality dietary protein
   b. Enables ruminants to use NPN efficiently
      - Ruminants can be productive without a source of dietary true protein
   c. Animal can survive on low amounts of dietary protein by recycling N (as urea) back to rumen

Ruminant Classification of Nitrogen
- crude protein ->
- soluble protein ->
- rumen bypass ->
- undegradable/degradable intake protein (UIP/DIP) ->
- rumen degradable/undegradable protein (RDP/RUP)

Limits to RUP/RDP system
- no AA balance
- most feeds contain more RDP than RUP
- lack of optimal ruminal fermentation when replacement with too much RUP
- lack of AA uniformity in by product feeds with high RUP
Nitrogen Requirements for Ruminants

A. Bug requirements come from
   1) NPN (NH₃)
   2) Degradable protein (amino acids)
      Considered rumen degradable intake protein (RDP)

B. Animal requirements come from
   1) rumen undegradable intake protein (RUP)
   2) microbial protein

Factors affecting RDP

- Proportion of NPN vs true protein
- Physical and chemical characteristics of true protein
  - Proteins with extensive disulfide bonds - slowly degraded
  - glutelins, prolamines
  - Linear proteins - rapidly degraded
    - Albumins
  - Fraction 1 protein - primary soluble protein in forages
    - r1 ribulose 1,5 biphosphate carboxylase (albumin)
- Heat treatments
- Rate of passage from rumen
- Particle size

Why does RDP drive milk yield?

- Improvements in rumen fermentation
  - Microbial protein synthesis
  - Carbohydrate utilization
  - Fiber digestibility

Ex. RDP stimulates rumen digestibility

<table>
<thead>
<tr>
<th>Item</th>
<th>RDP, % of DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM*</td>
<td>50.2</td>
</tr>
<tr>
<td>NDF*</td>
<td>50.1</td>
</tr>
<tr>
<td>ADF</td>
<td>42.4</td>
</tr>
<tr>
<td>NSC*</td>
<td>83.1</td>
</tr>
</tbody>
</table>

* P < .05

Griswold et al., 2003
However, at the same level of RDP, adding urea further stimulates digestion

<table>
<thead>
<tr>
<th>Item</th>
<th>Low</th>
<th>High</th>
<th>% diff</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM*</td>
<td>45.3</td>
<td>59.1</td>
<td>30.4%</td>
</tr>
<tr>
<td>NDF*</td>
<td>50.4</td>
<td>52.6</td>
<td>4.5%</td>
</tr>
<tr>
<td>ADF*</td>
<td>39.9</td>
<td>46.4</td>
<td>16.3%</td>
</tr>
<tr>
<td>NSC*</td>
<td>78.5</td>
<td>92.8</td>
<td>18.2%</td>
</tr>
</tbody>
</table>

* P < .05 Griswold et al., 2003

Why? Fiber digesting bacteria love NPN!

RDP stimulates NDF digestion

Stokes et al., 1991; 37% NSC treatment

Digestible intake protein (DIP) and microbial efficiency.

Getting the balance right is important: Interaction between RUP, RDP and milk yield

NRC, 2001

RDP vs. RUP – have we gone too far?

- Significant increases in RUP feeding
- Did it improve performance?

Review of RUP – Milk yield

- In 127 comparisons from 88 lactation trials,
  - Milk yield was significantly higher in only 17% of comparisons
  - Milk protein % increased in 5% of comparisons, decreased in 22%
  - SBM vs. bypass soy ingredients
    - Bypass increased milk in 6 of 29 comparisons
    - Milk protein decreased in 8 of 29 comparisons

Santos et al., 1998
Why the lack of response to RUP?

- Poor AA quality of RUP supplements
- Poor digestibility of RUP supplements
- Decreased microbial protein production due to shortage of RDP
- Decreased carbohydrate utilization in the rumen due to shortage of RDP
- Decreased fiber digestion due to shortage of RDP

Over Replacement of Soy Peptides on Ruminal Fermentation

<table>
<thead>
<tr>
<th>Item</th>
<th>Peptide added as soy peptide, % of total N</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH₃, mg/100ml</td>
<td>8.38 7.08 5.14 3.94</td>
</tr>
<tr>
<td>MeN, g/day</td>
<td>1.18 1.37 1.32 1.24</td>
</tr>
<tr>
<td>MeN, G/kg DOM</td>
<td>25.0 26.8 26.8 28.8</td>
</tr>
<tr>
<td>VFAMN, mmol/kg</td>
<td>395.1 392.9 394.3 383.1</td>
</tr>
<tr>
<td>DOM, %</td>
<td>64.3 67.9 65.3 63.3</td>
</tr>
<tr>
<td>ADFd, %</td>
<td>39.6 39.1 36.3 32.1</td>
</tr>
<tr>
<td>NDFd, %</td>
<td>49.1 49.6 45.5 42.2</td>
</tr>
</tbody>
</table>

Ammonia is the Central N Compound in the Rumen – Factors affecting AMMONIA concentrations in the rumen

- Time of feeding
  - peak 1-2 hr w/traditional NPN, 3-4 hr w/protein
- Type of protein
- Synergy with CHO and energy
- Location (lower in mat than liquid)

Bacterial Requirements: 1) AMMONIA

1. NH₃ addition to the pool in rumen:
   - a. dietary protein degradation
   - b. dietary NPN
   - c. recycled urea: saliva and rumen wall
   - d. recycling of microbes

2. NH₃ disappearance:
   - a. incorporation into microbial protein
   - b. wash out
   - c. absorption into rumen wall

Ammonia requirements for bacterial growth

- 25 to 100% of microbial N from ammonia
- Maximal rate of fermentation in vivo:
  - 5 mg % Satter and Slyter, 1974 Brit. J. Nutr. 32.199
- Most bacteria have ammonia saturation constants <1.5 mg% thus 95% maximal growth at 1mM which is less than shown in vivo.
- Why are there differences?
  - diurnal variation
  - micro environment
Effect of Dietary Protein on Variation in Rumen NH3

<table>
<thead>
<tr>
<th>Protein</th>
<th>Rumen NH3, mg%</th>
<th>% of Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>&lt; 3</td>
<td>80</td>
</tr>
<tr>
<td>11</td>
<td>&lt; 3</td>
<td>50</td>
</tr>
<tr>
<td>14</td>
<td>&lt; 3</td>
<td>20</td>
</tr>
</tbody>
</table>

Diurnal ammonia variation in TMR fed cows
(Cows fed 6X – pushed up 8X)

Major Users of Rumen NH3
Fibrolytic Bacteria

- Nitrogen metabolism
  - cannot use amino acids for making body protein
  - thus they need: NH3 (source of N) and carbon skeletons (back bone of amino acid)

Degradation of Protein in the Rumen to Make NH3-N and Carbon Skeletons
Degradation of feed protein by bacteria:

- \( \text{NH}_2 \) protein \( \rightarrow \) Free AA \( \rightarrow \) valine
- CH3-CH-CH-COOH
- peptides
- CH3
- NH3 deamination
- isobutyric acid
- CH3-CH-C-COOH
- CH3
- CH3
- decarboxylation

Use of BCAA and NH3 by Fibrolytics to Make Essential Amino Acids (EAA)

Use of BCAA by Fibrolytics to Make EAA
**Use of BCAA by Fibrolytics to Make EAA**

- Valeric to proline: \( \text{CH}_3\text{-CH}_2\text{-CH}_2\text{-CH}_2\text{-COOH} \rightarrow \text{proline} \)
- 2 methyl butyrate to isoleucine: \( \text{CH}_3\text{-CH}_2\text{-CH-} \rightarrow \text{isoleucine} \)

**Ammonia Must Be Assimilated by Bacteria**

\[
\begin{align*}
\text{NH}_3 + 2\text{ketoglutarate} & \rightarrow \text{Glutamate} \\
\text{GDH} & \rightarrow \text{Glutamine} \\
\text{GDH} & \rightarrow \text{Glutamate dehydrogenase; GS=glutamine synthetase; GOGAT=glutamate synthase} \\
\text{NAD}^+ & \rightarrow \text{NADPH} \\
\text{NADH} & \rightarrow \text{NAD}^+ \\
\text{ATP} & \rightarrow \text{ADP} + \text{Pi}
\end{align*}
\]

**Goals for Supplying Nitrogen for the Bugs**

1. Maximize bacterial protein from non-protein nitrogen (NPN)
2. Minimize ruminal degradation of true protein for that which is needed to maximize microbial protein

**How much microbial N or CP is made in the rumen?**

- **Microbial N, grams/d**
  - 0 grams/d
  - 5 grams/d
  - 10 grams/d
  - 20 grams/d
  - 30 grams/d
  - 50 grams/d
  - 100 grams/d
  - 200 grams/d
  - 300 grams/d
  - 400 grams/d
  - 500 grams/d

**Total Tract Digestion of Organic Matter, kg/d**

- 0 kg/d
- 5 kg/d
- 10 kg/d
- 15 kg/d
- 20 kg/d
- 25 kg/d

**What is Microbial Protein Worth?**

- A human consuming a 10-ounce steak eats about 3 ounces of protein

**Rumen Bacteria Don't Care Where They Get Their Source of NH3-N From**

- But they do care how and when they see it

**Microbes produced in the rumen supply a cow the equivalent of 15-20, 10-oz steaks/day!**
Limitations of Microbial Protein Synthesis

- Three most major limitations
  - Energy available
  - NH₃ available
  - Carbon skeletons (e.g. branched chain fatty acids)

- Two most likely limitations
  - Energy available
  - NH₃ available
  - These need to be synchronized

Rumen NH₃ Following Protein Ingestion

Rumen VFA from Carbohydrate Sources

Matching Protein and Energy Sources

NPN - SOURCES

Traditional Urea:
- solid prill form
- common NPN supplement
- liquid feeds

Ammonia:
- anhydrous or aqua ammonia added to silage
- add to corn silage (35% DM):
  - 6-8 lb NH₃/ton
  - raises CP from about 8 to 12%
- difficult to work with

Urea -> Ammonia

\[ \text{Urea} = \text{CO(NH}_2\text{)} \]

Urease from rumen bacteria
Incorporation of NPN into diets

- Use of urea requires adaptation period 3 wks
- Best if in TMR
- Liquid supplements with rapidly degradable CHO
- Avoid slug feeding
- Avoid in diets with wet silages
- Must balance RDP/ RUP

Effect of rumen degraded protein and infused urea on microbial yield and fiber & starch & sugar digestion.

<table>
<thead>
<tr>
<th>VARIABLES</th>
<th>RDP, 8%DM</th>
<th>RDP, 11%DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH₃, mg/100ml</td>
<td>No Urea</td>
<td>Plus Urea</td>
</tr>
<tr>
<td></td>
<td>0.28</td>
<td>6.26</td>
</tr>
<tr>
<td>Microbial N, g/day</td>
<td>No Urea</td>
<td>Plus Urea</td>
</tr>
<tr>
<td></td>
<td>1.09</td>
<td>2.71</td>
</tr>
<tr>
<td>Microbial N, g/kg CHO dig</td>
<td>No Urea</td>
<td>Plus Urea</td>
</tr>
<tr>
<td></td>
<td>24.1</td>
<td>35.5</td>
</tr>
<tr>
<td>ADP, %</td>
<td>No Urea</td>
<td>Plus Urea</td>
</tr>
<tr>
<td></td>
<td>47.5</td>
<td>52.7</td>
</tr>
<tr>
<td>NDF, %</td>
<td>No Urea</td>
<td>Plus Urea</td>
</tr>
<tr>
<td></td>
<td>47.5</td>
<td>52.7</td>
</tr>
<tr>
<td>Hemicellulose Digestion, %</td>
<td>No Urea</td>
<td>Plus Urea</td>
</tr>
<tr>
<td></td>
<td>55.8</td>
<td>61.6</td>
</tr>
<tr>
<td>Starch &amp; Sugar Digestion, %</td>
<td>No Urea</td>
<td>Plus Urea</td>
</tr>
<tr>
<td></td>
<td>73.6</td>
<td>92.5</td>
</tr>
</tbody>
</table>

1Adapted from Griswold et al, 2003

Nitroshure gradually supplies N to the rumen in a similar pattern to soybean meal (isonitrogenous amounts of N)

Ammonia production, in vitro

Impact of replacing Urea w/ Nitroshure (Artificial rumen study WVU)

- Nitroshure is coated with all natural, GRAS approved materials
- The controlled release technology used for Nitroshure is patented and therefore is a unique innovation for the feed industry

Impact of replacing SBM w/ Nitroshure (Artificial rumen study WVU)

Key pt: Nitroshure maintained the same total protein supply past the rumen by capturing more microbial protein. This difference would equal 0.63 lb/d more microbial protein in a cow eating 50 lb.
Changes in rumen performance in response to Nitroshure

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>Nitroshure</th>
<th>Diff</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM dig.</td>
<td>60.0%</td>
<td>65.6%</td>
<td>5.6%</td>
</tr>
<tr>
<td>NDF dig.</td>
<td>53.7%</td>
<td>59.4%</td>
<td>5.7%</td>
</tr>
<tr>
<td>CHO dig.</td>
<td>46.6%</td>
<td>50.7%</td>
<td>4.1%</td>
</tr>
</tbody>
</table>

Replaced 2.0 lbs. of SBM w/.37 lbs of Nitroshure, remainder a blend of corn meal and molasses.

Nitroshure

Nitroshure vs. urea
- Increases efficiency of N utilization by matching N supply to microbial needs
- Allows for feeding of higher concentrations of NPN safely

Nitroshure vs. true protein
- More efficient and effective at increasing and maintaining high rumen ammonia concentrations
- Maintains or improves protein supply to the cow
- Maintains or improves fiber and overall carbohydrate utilization by the rumen

Nitroshure is more efficient than true protein to supply the rumen w/ammonia

We must be cautious about excessive ammonia in the rumen

Excessive Protein Degradation or NH3 in the Rumen is...
- Wasteful because it lowers the amount of amino acids available for intestinal absorption
- Excess ammonia must be detoxified in the liver
  - requires 2 ATP per N

Excess ammonia must be detoxified in the liver
- Requires 4 ATP
Recommendations for Rumen Degradable Protein balancing

<table>
<thead>
<tr>
<th>Item</th>
<th>% DM</th>
<th>g per day (based on 23 kg DMI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RDP</td>
<td>11 to 12</td>
<td>2500 to 2700</td>
</tr>
<tr>
<td>Rapidly degraded N from feeds</td>
<td>2.5 to 3.5</td>
<td>64 to 96</td>
</tr>
<tr>
<td>Urea</td>
<td>0.2 to 0.55</td>
<td>48 to 723</td>
</tr>
<tr>
<td>Nitrobure</td>
<td>0.3 to 0.8</td>
<td>91 to 182</td>
</tr>
</tbody>
</table>

Summary

- RDP needs to be considered as an important part of the nutrition program in order to maximize effectiveness of protein nutrition.
- RDP sources differ in their utilization and should be blended much in the same manner we blend RUP sources.
- Encapsulated urea can increase the effectiveness of RDP nutrition by allowing for the safe and effective feeding of higher levels of urea.