Protein Nutrition of Ruminants – *Into the Future*

Chuck Schwab  
Schwab Consulting, LLC, Boscobel, Wisconsin  
Professor Emeritus, Animal Sciences  
University of New Hampshire
Presentation

• Brief review of ruminant protein nutrition
• Importance of managing carbohydrate nutrition for efficient and successful protein nutrition
• Importance of managing protein and AA nutrition for efficient and successful protein nutrition
• Role of rumen protected Met and Lys supplements for efficient protein nutrition
• Benefits of AA balancing
• Evaluating rumen protected AA supplements
• Summary and conclusions
Presentation

• Brief review of ruminant protein nutrition
• Importance of managing carbohydrate nutrition for efficient and successful protein nutrition
• Importance of managing protein nutrition and AA for efficient and successful protein nutrition
• Important role of rumen protected Met and Lys supplements for efficient protein nutrition
• Benefits of AA balancing
• Evaluating rumen protected AA supplements
• Summary and conclusions
Ruminant protein nutrition

- Crude protein
- Saliva
- True protein
- Peptides
- Ammonia
- NPN
- Microbial protein
- Urea
- Liver
- Amino acids
- Rumin
- RUP
- Microbial protein
- Endogenous protein
- Metabolizable protein
- Small intestine
- MILK
- Mammary gland
Rumen microorganisms have RDP requirements and animals have AA requirements

1) **RDP** – purpose is to meet the ammonia and other NPN requirements of *rumen microbes* for maximum carbohydrate digestion and synthesis of microbial protein

![Image of Rumen Microbes](image1.png)

2) **RUP** – purpose is to provide the additional AA that the *animal* requires that are not provided by microbial protein

![Image of Cattle](image2.png)
Presentation

• Brief review of ruminant protein nutrition
• Importance of managing carbohydrate nutrition for efficient and successful protein nutrition
  • Importance of managing protein and AA nutrition for efficient and successful protein nutrition
  • Important role of rumen protected Met and Lys supplements for efficient protein nutrition
• Benefits of AA balancing
• Evaluating rumen protected AA supplements
• Summary and conclusions
Optimizing carbohydrate nutrition maximizes yields of microbial protein and VFA
Effect of varying dietary protein and NFC on production of lactating cows

- Fed 3 levels of NFC (37, 41 and 46%) and 3 levels of CP (15.1, 16.7 and 18.4%) to 63 mid-lactation cows
- Diets were formulated from alfalfa and corn silages, rolled high-moisture shelled corn (HMC), soybean meal, minerals and vitamins
- Forage portion of the diets were 60% alfalfa, 40% corn silage on all diets
- NFC contents of 37, 41 and 46% were obtained by feeding 75, 63 and 50% forage
- Dietary CP contents of 15.1, 16.7 and 18.4% were obtained by replacing HMC with soybean meal
- Regarding the results, effects of dietary NFC were not confounded by dietary CP

Broderick (2003)
Effect of dietary CP on milk production and feed intake

<table>
<thead>
<tr>
<th>Item</th>
<th>CP, % of DM</th>
<th>P &gt; F</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15.1</td>
<td>16.7</td>
<td>18.4</td>
<td>CP</td>
</tr>
<tr>
<td>DMI, kg/d</td>
<td>21.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>22.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>22.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Milk, kg/d</td>
<td>33.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>34.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>34.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.01</td>
</tr>
<tr>
<td>Fat, kg/d</td>
<td>1.15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Milk/DMI</td>
<td>1.56&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.55&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.51&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.02</td>
</tr>
<tr>
<td>Milk N/N intake</td>
<td>0.30&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.27&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.24&lt;sup&gt;c&lt;/sup&gt;</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>MUN, mg/dl</td>
<td>9.2</td>
<td>12.4</td>
<td>15.9</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Urea N/NPN, %</td>
<td>31.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>38.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>43.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Broderick (2003)
Effect of dietary CP on nutrient digestibility and N metabolism

<table>
<thead>
<tr>
<th>Item</th>
<th>CP, % of DM</th>
<th>P &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Linear</td>
</tr>
<tr>
<td>NDF dig, %</td>
<td>37.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>37.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fecal DM, kg/d</td>
<td>7.95</td>
<td>8.25</td>
</tr>
<tr>
<td>Fecal N, g/d</td>
<td>236&lt;sup&gt;b&lt;/sup&gt;</td>
<td>264&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Urine, L/d</td>
<td>20.8&lt;sup&gt;c&lt;/sup&gt;</td>
<td>24.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Urinary N, g/d</td>
<td>140&lt;sup&gt;c&lt;/sup&gt;</td>
<td>193&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Urinary PD&lt;sup&gt;1&lt;/sup&gt;, mmol/d</td>
<td>377&lt;sup&gt;b&lt;/sup&gt;</td>
<td>404&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup> PD = total purine derivatives (allantoin plus uric acid)

Broderick (2003)
## Effect of dietary NFC on milk production and feed intake

<table>
<thead>
<tr>
<th>Item</th>
<th>NFC, % of DM</th>
<th>P &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>NFC Linear</td>
</tr>
<tr>
<td>BW gain, kg/d</td>
<td>0.39&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>0.47&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.71&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Milk, kg/d</td>
<td>31.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>33.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>36.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Fat, %</td>
<td>3.86&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>3.70&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.22&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Protein, %</td>
<td>2.74&lt;sup&gt;c&lt;/sup&gt;</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>2.80&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.87&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Lactose, %</td>
<td>4.92&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>4.97&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.99&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Milk/DMI</td>
<td>1.44&lt;sup&gt;c&lt;/sup&gt;</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>1.53&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.65&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Milk N/N intake</td>
<td>0.25&lt;sup&gt;c&lt;/sup&gt;</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>0.27&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.30&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>MUN, mg/dl</td>
<td>13.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>12.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>11.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

*Broderick (2003)*
Effect of dietary NFC on nutrient digestibility and N metabolism

<table>
<thead>
<tr>
<th>Item</th>
<th>NFC, % of DM</th>
<th>P &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NFC</td>
<td>Linear</td>
</tr>
<tr>
<td>OM dig, %</td>
<td>63.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>63.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>65.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>NDF dig, %</td>
<td>40.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>38.0&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>36.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Fecal DM, kg/d</td>
<td>8.37&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>8.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>7.88&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.02</td>
</tr>
<tr>
<td>Urinary N, g/d</td>
<td>200&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>189&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>180&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Urinary N, % of intake</td>
<td>34.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>31.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>30.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Urinary PD, mmol/d</td>
<td>356&lt;sup&gt;c&lt;/sup&gt;</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>399&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>425&lt;sup&gt;c&lt;/sup&gt;</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Broderick (2003)
Starch type also affects the AA status of lactating dairy cows

Starch type affects site, rate and extent of starch digestion

- Cereal grain type [e.g., barley, corn, oats, sorghum, wheat]
- Endosperm type (e.g., floury vs flinty corn) and maturity
- Grain storage & processing [(dry, high moisture or ensiled), (cracked, rolled or ground) or steam-flaked]
Effect of enhanced starch nutrition on the AA status of lactating cows…a summary

- Increased milk yield
- Increased milk protein content
- Increased milk protein yield
- Increased milk/DM intake ratios
- Increased milk N/N intake ratios
- Decreased milk urea
- Decreased urinary N excretion
- Decreased milk fat content

- Increased VFA synthesis
- More microbial protein
- More efficient microbial protein
- Increased AA passage to SI
- Lower rumen ammonia
- Increased net portal appearance of total AA, oxygen, glucose, butyrate and insulin
Right blend of carbohydrates…and does digestibility matter?

PLANT CARBOHYDRATES

Cell contents
- Organic acids
- Sugars (glucose, fructose, sucrose, lactose)
- Starch
- Fructans
- Pectic substances and B-glucan

Cell walls
- Hemicellulose
- Cellulose

Soluble fiber
Non-fiber carbohydrates (NFC)

ADF
NDF
Many researchers have demonstrated the importance of optimizing intakes of digestible fiber, starch and sugars on:

- Increasing microbial protein synthesis
- Increasing AA passage to the small intestine
- Increasing the capture of recycled N
- Making better use of rumen ammonia

Because of the profound effects that dietary carbohydrates can have on optimizing rumen function and AA nutrition:

- Feed testing labs will continue to provide more detailed analysis of individual carbohydrates in feeds and their digestibility
- Modelers will continue to seek ways to more accurately predict microbial protein synthesis from dietary carbohydrate supply
Presentation

- Brief review of ruminant protein nutrition
- Importance of managing carbohydrate nutrition for efficient and successful protein nutrition
- Importance of managing protein nutrition and AA for efficient and successful protein nutrition
  - Important role of rumen protected Met and Lys supplements for efficient protein nutrition
- Benefits of AA balancing
- Evaluating rumen protected AA supplements
- Summary and conclusions
N excretion of lactating dairy cows fed energy limiting diets and different levels of dietary CP (14.0 to 18.7%) (average milk yield = 40 kg)

Van Amburgh et al. (2015)
Rumen microorganisms have RDP requirements and animals have AA requirements

1) **RDP** – purpose is to meet the ammonia and other NPN requirements of *rumen microbes* for maximum carbohydrate digestion and synthesis of microbial protein

2) **RUP** – purpose is to provide the additional AA that the *animal* requires that are not provided by microbial protein
Amino acids are the required nutrients

**Essential**
1. Arginine
2. Histidine
3. Isoleucine
4. Leucine
5. Lysine
6. Methionine
7. Phenylalanine
8. Threonine
9. Tryptophan
10. Valine

**Non-essential**
1. Alanine
2. Aspartic acid
3. Asparagine
4. Cysteine
5. Glutamic acid
6. Glutamine
7. Glycine
8. Proline
9. Serine
10. Tyrosine
Amino acids have numerous metabolic functions

- Tissue proteins
- Enzymes
- Messenger proteins
- Receptor proteins
- Ion channel proteins
- Milk proteins
- Blood proteins

Connective
Epithelial
Nervous
Muscle

Protein synthesis

Albumins
Globulins
Fibrinogen
Regulatory proteins
Clotting factors

Metabolic regulation

NPN compounds

Nitrogenous bases
Creatinine/creatine
Histamine
Polyamines, etc.

Amino Acids

Energy

- Glucose
- Fat

Energy
# Amino acids are the required nutrients

**Essential**

1. Arginine
2. Histidine
3. Isoleucine
4. Leucine
5. Lysine
6. Methionine
7. Phenylalanine
8. Threonine
9. Tryptophan
10. Valine
# Amino acids are the required nutrients

<table>
<thead>
<tr>
<th>Essential</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Arginine</td>
</tr>
<tr>
<td>2. Histidine</td>
</tr>
<tr>
<td>3. Isoleucine</td>
</tr>
<tr>
<td>4. Leucine</td>
</tr>
<tr>
<td>5. Lysine</td>
</tr>
<tr>
<td>6. Methionine</td>
</tr>
<tr>
<td>7. Phenylalanine</td>
</tr>
<tr>
<td>8. Threonine</td>
</tr>
<tr>
<td>9. Tryptophan</td>
</tr>
<tr>
<td>10. Valine</td>
</tr>
</tbody>
</table>

- **Not** crude protein
- **Not** metabolizable protein
- **It's** amino acids
- **It's** the supply of the most limiting EAA that determines animal productivity
Amino acids are the required nutrients

**Essential**
1. Arginine
2. **Histidine**
3. Isoleucine
4. Leucine
5. **Lysine**
6. **Methionine**
7. Phenylalanine
8. Threonine
9. Tryptophan
10. Valine

- Not crude protein
- Not metabolizable protein
- It's amino acids
- It's the supply of the most limiting EAA that determines animal productivity
Milk yield (kg/d) = 0.8 X DMI (kg/d) + 2.3 X CP (%) – 0.05 X CP² (%) – 9.8

(r² = 0.29)

No correlation between DMI and %CP; dietary CP and milk protein %
$y = 0.972x + 7.2309$

$R^2 = 0.7212$

VT Dairy Farm Sustainability Project, 2002
\[ y = 0.4524x - 62.063 \]
\[ R^2 = 0.74 \]

\[ y = -0.3497x^2 + 55.631x - 732.68 \]
\[ R^2 = 0.81 \]

\[ y = -0.0195x^2 + 13.098x - 457.31 \]
\[ R^2 = 0.92 \]

Schwab et al. (2003, 2004)
Differences between actual milk and MP allowable milk and predicted Lys in MP using NRC (2001)

\[ y = 5.6532x - 35.074 \]

\[ R^2 = 0.2135 \]

(n = 63 of 206)
Differences between actual milk and MP allowable milk and predicted Lys in MP using NRC (2001)

\[(n = 81 \text{ of } 206)\]

\[y = 21.673x - 41.357\]

\[R^2 = 0.2051\]

UNH Data Set, 2002
Amino acid balancing

Definition

A deliberate attempt, through selective use of protein supplements and RP-AA supplements, to achieve an amount and profile of absorbed AA that comes as close as possible to meeting the cows requirements for optimal health and performance without wasteful excesses.
Amino acid balancing

Definition

A deliberate attempt, through selective use of protein supplements and RP-AA supplements, to achieve an amount and profile of absorbed AA that comes as close as possible to meeting the cows requirements for optimal health and performance without wasteful excesses

What is the optimal balance?
Optimum content of Lys in MP (NRC, 2001)

Milk protein content responses, g/100 g

Percent Lys in MP (Met > 1.95 of MP)
Optimum content of Met in MP (NRC, 2001)

Milk protein content responses, (g/100 g)

Optimum content of Met in MP (NRC, 2001)
Optimum Lys and Met concentrations in MP for maximal content of milk protein

<table>
<thead>
<tr>
<th>Model</th>
<th>Lys</th>
<th>Met</th>
<th>Optimal Lys/Met ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>NRC (2001)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Original release¹</td>
<td>6.80</td>
<td>2.29</td>
<td>2.97</td>
</tr>
<tr>
<td>Revised v.1.1.9³</td>
<td>6.83</td>
<td>2.28</td>
<td>3.00</td>
</tr>
<tr>
<td>CPM-Dairy²</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prior v6.1²</td>
<td>6.68</td>
<td>2.40</td>
<td>2.78</td>
</tr>
<tr>
<td>v6.1³</td>
<td>6.97</td>
<td>2.53</td>
<td>2.75</td>
</tr>
<tr>
<td>v6.5⁴</td>
<td>6.77</td>
<td>2.85</td>
<td>2.37</td>
</tr>
</tbody>
</table>

¹ Schwab et al. (2009), ² Whitehouse et al. (2009), ³ Whitehouse et al. (2013), ⁴ Foskolos et al. (2014)
Presentation

• Brief review of ruminant protein nutrition
• Importance of managing carbohydrate nutrition for efficient and successful protein nutrition
• Importance of managing protein and AA nutrition for efficient and successful protein nutrition
• Important role of rumen protected Met and Lys supplements for efficient protein nutrition
  • Benefits of AA balancing
  • Evaluating rumen protected AA supplements
  • Summary and conclusions
## Ruminant Lys and Met supplements in North America

**Lys supplements**
- AjiPro® -L
- AminoShure-L
- Bovi-Lysine
- Lysine 35™
- LysiPEARL™
- Megamine-L™
- MetaboLys®
- NoviLys®
- USA Lysine™

**Met supplements**
- Smartamine M®
- Mepron® M85
- AminoShure-M
- MetaboMet™
- MethioPlus™
- MetiPEARL™
- Novimet®

**Met analogs**
- MetaSmart® (HMBi)
- Alimet®
- Rhodimet AT 88®
- MFP™ (CaMHA)
• Brief review of ruminant protein nutrition
• Importance of managing carbohydrate nutrition for efficient and successful protein nutrition
• Importance of managing protein and AA nutrition for efficient and successful protein nutrition
• Important role of rumen protected Met and Lys supplements for efficient protein nutrition
• Benefits of AA balancing
  • Evaluating rumen protected AA supplements
  • Summary and conclusions
Benefits of AA balancing in lactating dairy cows in the US

1. Reduced RUP requirements for higher milk yield and milk component concentrations (1.5 to 2.0% units less of DM)
2. Increased milk yield, particularly in early lactation cows (2 to 4.0 kg/d more milk are common)
A summary of some early lactation and transition cow experiments

<table>
<thead>
<tr>
<th>Week of lactation</th>
<th>RPAA used</th>
<th>Conducted by</th>
<th>Milk, kg/d</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cont</td>
</tr>
<tr>
<td>0 - 8</td>
<td>LM</td>
<td>Julien et al. (1999)</td>
<td>45.7</td>
</tr>
<tr>
<td>0 - 6</td>
<td>LM</td>
<td>Robinson et al. (1996)</td>
<td>33.8</td>
</tr>
<tr>
<td>0 - 4</td>
<td>LM</td>
<td>Sniffen et al. (1999)</td>
<td>43.4</td>
</tr>
<tr>
<td>0 - 6</td>
<td>L, LM</td>
<td>Sniffen et al. (1999)</td>
<td>42.9</td>
</tr>
<tr>
<td>0 - 6</td>
<td>L</td>
<td>Nocek et al. (1999)</td>
<td>37.1</td>
</tr>
<tr>
<td>0 - 4</td>
<td>LM</td>
<td>Chalupa et al. (1999)</td>
<td>32.6</td>
</tr>
<tr>
<td>0 - 10</td>
<td>LM</td>
<td>Harrison et al. (1995)</td>
<td>34.7</td>
</tr>
<tr>
<td>0 - 4</td>
<td>L</td>
<td>Nocek et al. (2011)</td>
<td>37.2</td>
</tr>
</tbody>
</table>

Ave. Milk response = 3.2 kg
Benefits of AA balancing in lactating dairy cows in the US

1. Reduced RUP requirements for higher milk yield and milk component concentrations (1.5 to 2.0% units less of DM)
2. Increased milk yield, particularly in early lactation cows (2 to 4.0 kg/d more milk are common)
3. Increased milk component concentrations (0.10 to 0.20% unit increases in protein and 0.10 to 0.15% unit increases in fat are common)
4. Healthier transition cows
5. Growing evidence of larger embryos, healthier embryos, reduced embryonic loss, and better breeding
6. Increased herd profitability
Body condition score (BCS), DM intake, milk yield and milk composition\(^1\)

<table>
<thead>
<tr>
<th></th>
<th>Diet</th>
<th>P-value</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CON</td>
<td>RPHMI</td>
<td>RPMet</td>
</tr>
<tr>
<td>DMI, kg/d</td>
<td>13.3</td>
<td>15.2</td>
<td>15.6</td>
</tr>
<tr>
<td>Milk, kg/d</td>
<td>35.7(^b)</td>
<td>38.1(^{ab})</td>
<td>40.0(^a)</td>
</tr>
<tr>
<td>Milk protein, %</td>
<td>3.04(^b)</td>
<td>3.26(^a)</td>
<td>3.19(^{ab})</td>
</tr>
<tr>
<td>Milk fat, %</td>
<td>4.27</td>
<td>4.68</td>
<td>4.09</td>
</tr>
<tr>
<td>ECM, kg/d</td>
<td>41.0(^b)</td>
<td>44.8(^a)</td>
<td>45.0(^a)</td>
</tr>
</tbody>
</table>

\(^1\) Osorio et al. (2013)

\(^2\) Contrast statement of CON versus MS + SM
Biomarkers analyzed in blood and liver tissue

**Metabolism**
- Urea
- Creatinine
- NEFA
- BHBA
- VLDL

**Liver function**
- Gamma-glutamyl transpeptidase (GGT)
- Glutamic oxaloacetic transaminase (GOT)
- Albumin
- Cholesterol
- Bilirubin
- Total lipid
- TAG
- Carnitine

**Oxidative stress**
- Paraoxonase
- Total antioxidant status
- Reactive oxygen metabolites
- Nitrates
- Retinol
- Tocopherol (Vit. E)
- Beta-carotene
- Glutathione

**Inflammation**
- Haptoglobin
- Ceruloplasmin
- Serum amyloid A
- IL-6

**Liver tissue**
## Biomarkers of liver function, inflammation and oxidative stress

<table>
<thead>
<tr>
<th></th>
<th>Diet</th>
<th>P-value</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CON</td>
<td>RPHMB</td>
<td>RPMet</td>
<td>Diet</td>
<td>Met²</td>
</tr>
<tr>
<td><strong>Liver function</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carnitine, nmol/g tiss.</td>
<td>37.5</td>
<td>98.2</td>
<td>66.0</td>
<td>0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Albumin, g/L</td>
<td>35.1</td>
<td>36.1</td>
<td>35.7</td>
<td>0.28</td>
<td>0.15</td>
</tr>
<tr>
<td><strong>Inflammation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ceruloplasmin, umol/L</td>
<td>3.02</td>
<td>2.68</td>
<td>2.71</td>
<td>0.03</td>
<td><strong>0.009</strong></td>
</tr>
<tr>
<td>Serum amyloid A, ug/mL</td>
<td>61.0</td>
<td>40.7</td>
<td>43.5</td>
<td>0.17</td>
<td>0.06</td>
</tr>
<tr>
<td><strong>Oxidative stress</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ORAC, mol/L</td>
<td>11.9</td>
<td>12.9</td>
<td>12.4</td>
<td>0.05</td>
<td><strong>0.04</strong></td>
</tr>
<tr>
<td>Glutathione, mM</td>
<td>1.27</td>
<td>1.55</td>
<td>1.73</td>
<td>0.15</td>
<td>0.07</td>
</tr>
</tbody>
</table>

1 Osorio et al. (2014a)
2 Contrast statement of CON versus RPHMB + RPMet
Results and Conclusions

Supplementation with RPHMB or RPMet, when Lys was adequate:

- Increased milk production and milk protein content
- Increased post-calving DM intake
- Reduced liver lipid accumulation
- Increased blood phagocytosis (leukocyte-killing capacity)
- Tendency for reduced incidence of ketosis
- Increased biomarkers reflective of improved liver function
- Decreased biomarkers of inflammation
- Increased biomarkers reflective of reduced oxidative stress

Author conclusions: The beneficial effect of feeding RPHMB or RPMet on improved milk production was due, at least in part, to increased voluntary DMI, better immuno-metabolic status, and perhaps by optimizing the use of body lipid reserves

Osorio et al. (2013, 2014ab)
Methionine is unique by being involved in 1-carbon metabolism

- Microbial protein + RUP
  - Met
    - Folates (THF)
    - 5mTHF
    - DMGly
    - Betaine
    - Choline
    - Homocysteine
    - Cystathionine
    - Cysteine
  - SAM
    - CH₃ (methyl group)
    - Creatine
    - Phosphatidycholine
    - Choline
    - VLDL
    - DNA methylation (epigenetics)
    - Sarcosine
    - SAH
    - Choline
    - Glutathione
    - Taurine
    - Antioxidants
DL-MET and HMB in the Hepatocyte

- Met needs of the cell must be met by dietary sources or regeneration
- Liver can convert D-Met and HMB to L-Met (active form)
- Liver responds similarly to DL-Met and HMB
- Optimum Lys:Met ratio in MP (e.g., 3/1 in NRC, 2001) can be met with methionine and/or RP-analogs

Bowen et al. (2015)
## Effect of treatments on DM intake

<table>
<thead>
<tr>
<th></th>
<th>RP-Met</th>
<th></th>
<th>RP-Choline</th>
<th></th>
<th>( P )-value</th>
<th></th>
<th></th>
<th>M x C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
<td>SEM</td>
<td>+</td>
<td>SEM</td>
<td>MET</td>
<td>CHO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prepartum</td>
<td>0</td>
<td>0.5</td>
<td>0</td>
<td>0.5</td>
<td>0.02</td>
<td>0.22</td>
<td>0.66</td>
<td></td>
</tr>
<tr>
<td>DMI, kg/d</td>
<td>12.9(^b)</td>
<td>13.3</td>
<td>13.9</td>
<td>0.5</td>
<td></td>
<td>0.22</td>
<td>0.66</td>
<td></td>
</tr>
<tr>
<td></td>
<td>14.3(^a)</td>
<td></td>
<td></td>
<td>0.5</td>
<td>0.02</td>
<td>0.88</td>
<td>0.78</td>
<td></td>
</tr>
<tr>
<td>Post-partum</td>
<td>17.2(^b)</td>
<td>18.2</td>
<td>18.1</td>
<td>0.77</td>
<td>0.04</td>
<td>0.88</td>
<td>0.78</td>
<td></td>
</tr>
<tr>
<td>DMI, kg/d</td>
<td>19.0(^a)</td>
<td></td>
<td></td>
<td>0.77</td>
<td>0.04</td>
<td>0.88</td>
<td>0.78</td>
<td></td>
</tr>
</tbody>
</table>

Zhou et al. (2015)
## Effect of treatments on milk yield and milk composition

<table>
<thead>
<tr>
<th></th>
<th>RP-Met</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>P-value</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>+</td>
<td>SEM</td>
<td>0</td>
<td>+</td>
<td>SEM</td>
<td>MET</td>
<td>CHO</td>
<td>M x C</td>
</tr>
<tr>
<td>Milk, kg/d</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>40.0(^b)</td>
<td>44.0(^a)</td>
<td>1.54</td>
<td>42.5</td>
<td>41.5</td>
<td>1.52</td>
<td>0.03</td>
<td>0.56</td>
<td>0.91</td>
</tr>
<tr>
<td>ECM, kg/d</td>
<td></td>
<td></td>
<td></td>
<td>40.7(^b)</td>
<td>44.7(^a)</td>
<td>1.23</td>
<td>&lt;0.01</td>
<td>0.57</td>
<td>0.40</td>
</tr>
<tr>
<td>Protein, %</td>
<td></td>
<td></td>
<td></td>
<td>3.13(^b)</td>
<td>3.32(^a)</td>
<td>0.05</td>
<td>&lt;0.01</td>
<td>0.32</td>
<td>0.41</td>
</tr>
<tr>
<td>Protein, kg/d</td>
<td></td>
<td></td>
<td></td>
<td>1.24(^b)</td>
<td>1.43(^a)</td>
<td>0.04</td>
<td>&lt;0.01</td>
<td>0.70</td>
<td>0.58</td>
</tr>
<tr>
<td>Fat, %</td>
<td>3.75</td>
<td>3.74</td>
<td>0.14</td>
<td>3.74</td>
<td>3.77</td>
<td>0.14</td>
<td>0.92</td>
<td>0.84</td>
<td>0.28</td>
</tr>
<tr>
<td>Fat, kg/d</td>
<td>1.43(^b)</td>
<td>1.58(^a)</td>
<td>0.04</td>
<td>1.52</td>
<td>1.50</td>
<td>0.04</td>
<td>0.02</td>
<td>0.76</td>
<td>0.15</td>
</tr>
<tr>
<td>MUN, mg/dL</td>
<td>12.87</td>
<td>12.89</td>
<td>0.42</td>
<td>12.68</td>
<td>13.08</td>
<td>0.42</td>
<td>0.96</td>
<td>0.29</td>
<td>0.57</td>
</tr>
</tbody>
</table>

Zhou et al. (2015)
Supplementation with RP-Met
- Increased DMI before and after calving
- Reduced incidence of ketosis
- Increased milk and milk component yields
- Increased milk protein concentrations
- Increased neutrophil phagocytosis and monocyte oxidative burst
- Increase plasma albumin (negative APP), decreased haptoglobin (positive APP), and increased paraoxonase (antioxidant)

Supplementation with RP-Choline
- Increased plasma glucose and decreased plasma creatinine
- Increased monocyte oxidative burst

Conclusions: Feeding RP-Met improved milk production, at least in part, because of increased DMI, and improved liver function and immune function

Zhou et al. (2015)
Presentation

• Brief review of ruminant protein nutrition
• Importance of managing carbohydrate nutrition for efficient and successful protein nutrition
• Importance of managing protein and AA nutrition for efficient and successful protein nutrition
• Important role of rumen protected Met and Lys supplements for efficient protein nutrition
• Benefits of AA balancing
• Evaluating rumen protected AA supplements
• Summary and conclusions
Ruminant Lys and Met supplements in North America

**Lys supplements**
- AjiPro® -L
- AminoShure-L
- Bovi-Lysine
- Lysine 35™
- LysiPEARL™
- Megamine-L™
- MetaboLys®
- NoviLys®
- USA Lysine™

**Met supplements**
- Smartamine M®
- Mepron® M85
- AminoShure-M
- MetaboMet™
- MethioPlus™
- MetiPEARL™
- Novimet®
- MetaSmart® (HMBi)
- Alimet®
- Rhodimet AT 88®
- MFP™ (CaMHA)

**RP-Lys**

**RP-Met**

**Met analogs**
Ruminant Lys and Met supplements in North America

**Lys supplements**
- AjiPro® -L
- AminoShure-L
- Bovi-Lysine
- Lysine 35™
- LysiPEARL™
- Megamine-L™
- MetaboLys®
- NoviLys®
- USA Lysine™

**Met supplements**
- Smartamine M®
- Mepron® M85
- AminoShure-M
- MetaboMet™
- MethioPlus™
- MetiPEARL™
- Novimet®
- MetaSmart® (HMBi)

- Alimet®
- Rhodimet AT 88®
- MFP™ (CaMHA)
Adoption of AA balancing in the United States

N° of cows fed AA balanced rations (OOOs)

![Graph showing the adoption of AA balancing in the United States from 2000 to 2014. The number of cows fed AA balanced rations increases over the years, reaching a peak of 1,430 in 2014.](image)

Adisseo, 2015
Disappointments and frustrations exist in using RPAA supplements to balance rations for AA
Creating highly effective RPAA supplements is difficult

Facts:

- Small differences in manufacturing process and composition can have significant effects on product stability and product efficacy
  - Initial attempts to develop products begin in the late 1960's
  - A few companies have spent nearly 30 years refining their techniques
- Most products have limited research on product efficacy (how much of the AA is absorbed when handled and fed under commercial practices). Considerations:
  - Effect of “off-farm” handling and mixing practices on product stability
  - Effect of “on-farm” feeding practices (mixing, extended exposure to diets, etc.) on product stability
  - Resistance to ruminal degradation after exposed to the above
  - Intestinal release for absorption after exposed to the above
- **Point being:** The challenge is great, available products are not created equal, and the industry lacks a “gold standard method” for determining product efficacy
Some factors to consider when selecting your source of “protected AA”

1. Product concentration
2. Stability (storage, handling, mixing, exposure to diets and total mixed rations, etc.)
3. Rumen protection
4. Intestinal release and absorption
5. Confidence in supplier information
   - Company’s experience and history with RPAA
   - Company’s support of AA research
   - Methods used to evaluate product efficacy (production studies or studies designed to answer questions about stability, rumen protection, intestinal release, intestinal absorption?)
   - Availability of efficacy data

Cost per gram of absorbed AA
Methods for determining efficacy of RPAA supplements

- **In vitro**
  - Ammonia release
  - Amino acid release (modified 3-step method)
    - In rumen buffer
    - In abomasal buffer
    - In intestinal buffer
  - Amino acid release (Cornell/Ross assay)

- **In situ**
  - Amino acid loss from rumen and intestinal bags

- **In vivo**
  - Plasma free AA “area-under-the curve” (AUC) approach
  - Milk protein dose-response approach
  - HP-Arg approach (measurement of ruminal and intestinal disappearance)
  - Plasma free AA dose-response approach
## Procedural shortcomings

<table>
<thead>
<tr>
<th>Method</th>
<th>Shortcomings</th>
</tr>
</thead>
<tbody>
<tr>
<td>In vitro</td>
<td>▪ No influence of animal effects of rumination, etc.</td>
</tr>
</tbody>
</table>
| In situ - Dacron bag          | ▪ Effects of eating and rumination not considered  
▪ Disappearance from rumen bags means degradation  
▪ Passage rates are needed  
▪ Disappearance from mobile bags means absorption  
▪ Products subjected to hindgut digestion  
▪ Cannot be used with fine or soluble products |
| Plasma free AA - AUC          | ▪ Animals receive large pulse doses of product not otherwise encountered                                                              |
| Milk protein dose - response  | ▪ Must maintain AA deficiencies over range of dosages used                                                                               |
| Plasma free AA dose - response| ▪ No obvious ones at least for RP-Lys and Met supplements…linearity in response observed                                               |
Recommended procedure for using the plasma free AA dose-response approach for evaluating RPAA supplements

1. Obtain preliminary knowledge product stability (effect of “off-farm” and “on-farm handling and feeding practices”) before proceeding
2. Mix supplements with a small portion of the ration 8-12 h before feeding
3. Include all treatments in the same Latin square
4. Collect pretreatment data for covariate analysis
5. Minimum of 7-day experimental periods
6. Take several daily blood samples daily at a fixed time after feeding for a minimum of 3 consecutive days
7. Express plasma Lys as a percentage of total AA before calculating bioavailability
8. Express plasma Met or total sulfur AA on absolute concentration basis (ug/100 ml or uM) before calculating bioavailability

Whitehouse et al. (2016)
Changes in plasma free Lys concentrations with increasing amounts of infused or fed Lys

Bioavailability:
\[(0.0068/0.0183) \times 100 = 37\%\]

Whitehouse et al. (2012)
Changes in plasma free sulfur AA concentrations with increasing amounts of infused or fed Met

$y = 1.98x + 89.48$
$R^2 = 0.9936$

$y = 1.64x + 89.46$
$R^2 = 0.9829$

Bioavailability: $\frac{1.64}{1.98} = 0.828 \times 100 = 82.8\%$

**Infusion**

**RP-Met**

Two 5 x 5 replicates (2013, 2014)

Chirgwin et al. (2015)
A summary of 17 plasma AA dose-response experiments at the University of New Hampshire

<table>
<thead>
<tr>
<th>Type of RP-AA supplement</th>
<th># of experiments</th>
<th>Range in calculated estimates of metabolic availability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysine</td>
<td>13</td>
<td>5 to 87%</td>
</tr>
<tr>
<td>Methionine</td>
<td>4</td>
<td>11 to 83%</td>
</tr>
</tbody>
</table>

Whitehouse et al. (2015)
Summary

Two considerations of “ideal protein” for ruminant animals

1) **Quality of rumen degradable protein (RDP)**

   Known that:
   
   1) all forms of N contribute to microbial protein synthesis
   2) free AA and peptides are stimulatory to growth rate and yield of rumen microorganisms
   3) proportion of microbial protein derived from ammonia varies according to availability of other N sources
   4) ammonia can become limiting for OM digestion and microbial protein synthesis when dietary RDP is too low

2) **Profile of absorbed amino acids**

   Research indicates its seldom, if ever, ideal
Summary

World-wide, most producers and nutritionists still only consider CP when evaluating protein feeds and animal requirements.

The current trend of feeding lower CP diets, coupled with more precise feeding of RDP and increased AA balancing, will continue.

Motives:
- Reduced feed costs
- Higher and more efficient production
- Improved health and reproduction
- Reduced environmental impact

These feeding strategies will continue to be supported by research aimed at improving feed analysis and nutritional models, increasing efficiency of microbial protein synthesis and capturing of recycled N, and greater availability of proven RP-AA supplements.
Thank You