Impact of Feeding Amino Acids on Reproduction

Evolution of Milk Production and Reproduction in the Last 50 years

Embryonic and fetal losses from conception to term in lactating dairy cows
Reproduction is affected by events occurring earlier in lactation

<table>
<thead>
<tr>
<th>Health problem</th>
<th>Pregnant at day 30, %</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No clinical disease</td>
<td>66.9</td>
<td>--</td>
</tr>
<tr>
<td>Single clinical disease</td>
<td>56.5</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Multiple clinical disease</td>
<td>40.8</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>No subclinical disease</td>
<td>68.0</td>
<td>--</td>
</tr>
<tr>
<td>Single subclinical disease</td>
<td>63.6</td>
<td>0.36</td>
</tr>
<tr>
<td>Multiple subclinical disease</td>
<td>52.2</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Multiple factors affecting development of pre-antral follicles
Factors Affecting Pregnancy in Dairy Cows

1. Minimal body condition score before breeding
2. Select heat and inseminate at the correct time
3. Cervical and uterine inductions of high-quality oocytes
4. Have early and appropriate altrumation

Protein and Reproduction

- Negative association between urea N concentration and pregnancy in dairy cows when urea N is excessive
- Studies evaluating the effects of protein on embryo quality and pregnancy usually fed cows diets with excessive concentrations of true protein or urea

Protein (N) Utilization by the Ruminant

1. Bryant, J. and B. R. Moss, Montana State University
Influence of Dietary Ratio of RDP to RUP on Milk Urea Nitrogen (MUN)

MUN or PUN and Pregnancy per AI

Effect of Urea/Saline Infusion on Uterine pH on d 7-8 of the Estrous Cycle
Nitrogenous Compounds in Feeds

- True proteins
  - Polymers of amino acids (18 to 20 different amino acids) linked by peptide bonds
    - Essential amino acids (nondispensable)
      - Have to be present in the diet (absorbed)
      - Arg Lys Trp Leu Ile Val Met Thr Phe His
    - Nonessential amino acids (dispensable)
      - Synthesized in body tissues
      - Glu Gly Asp Pro Ala Ser Cys Tyr
- Proteins —— Peptides —— Amino acids

Amino Acids

- Building blocks of protein
- Contain an amino group (NH$_2$) and an acid group (COOH)
- Hooking amino acids together form a peptide
- Linking peptides form a protein

Amino Acid Composition, % Crude Protein

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Milk</th>
<th>Bact</th>
<th>Corn</th>
<th>Soy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cell wall</td>
<td>Non-wall</td>
<td>Mean</td>
<td></td>
</tr>
<tr>
<td>Methionine</td>
<td>1.97</td>
<td>2.71</td>
<td>2.40</td>
<td>2.68</td>
</tr>
<tr>
<td>Lysine</td>
<td>6.37</td>
<td>7.62</td>
<td>5.60</td>
<td>8.20</td>
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<tr>
<td>Histidine</td>
<td>2.47</td>
<td>2.74</td>
<td>1.74</td>
<td>2.69</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>3.53</td>
<td>4.75</td>
<td>4.20</td>
<td>5.16</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>0.49</td>
<td>1.51</td>
<td>NA</td>
<td>1.63</td>
</tr>
<tr>
<td>Threonine</td>
<td>3.90</td>
<td>3.72</td>
<td>3.30</td>
<td>5.59</td>
</tr>
<tr>
<td>Leucine</td>
<td>6.70</td>
<td>9.18</td>
<td>5.90</td>
<td>7.51</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>2.84</td>
<td>5.79</td>
<td>4.00</td>
<td>5.88</td>
</tr>
<tr>
<td>Valine</td>
<td>4.03</td>
<td>5.89</td>
<td>4.70</td>
<td>6.16</td>
</tr>
<tr>
<td>Arginine</td>
<td>3.30</td>
<td>3.40</td>
<td>3.82</td>
<td>6.96</td>
</tr>
</tbody>
</table>
Balances of Average Metabolizable Amino Acid for 16 Dairy California rations

20 farms selected

Some weekends and...

764 miles in a straight line

TMR Lactation Crude Protein (% DM)

<table>
<thead>
<tr>
<th>Region</th>
<th>Average</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>CENTRAL</td>
<td>16.75</td>
<td>12.3 – 19.7</td>
</tr>
<tr>
<td>NORTH</td>
<td>16.34</td>
<td>14.7 – 17.7</td>
</tr>
<tr>
<td>SOUTH</td>
<td>17.99</td>
<td>15.4 – 20.9</td>
</tr>
<tr>
<td>ALL</td>
<td>17.03</td>
<td>12.3 – 20.9</td>
</tr>
</tbody>
</table>
**MY COW**

- Holstein
- BW: 1,500 lbs.
- > 3 lactations
- 150 DIM
- 82 lbs. milk / day
  - 3.7% fat
  - 3.2% protein

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**Amino Acids and Reproduction**

- Bovine conceptus requires amino acids for development
  - Embryo growth
  - Placental development
- Some amino acids have physiological functions beyond building blocks for tissue deposition
  - Signaling molecules (Arginine -> NO, polyamines)
  - Neurotransmitter (GABA)
  - DNA methylation (Methionine)
Can Methionine Prevent Embryonic Losses?

Whole Rat Embryos Require Methionine for Placenta Tube Closure when Cultured on Cow Serum

<table>
<thead>
<tr>
<th>Cow serum with:</th>
<th>Embryo Protein</th>
<th>% Abnormal</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>73.7 ± 8.6</td>
<td>100%</td>
</tr>
<tr>
<td>Amino acids + vitamins</td>
<td>130.0 ± 7.2</td>
<td>0%</td>
</tr>
<tr>
<td>Amino acids</td>
<td>117.1 ± 6.5</td>
<td>0%</td>
</tr>
<tr>
<td>Vitamins</td>
<td>56.6 ± 5.7</td>
<td>100%</td>
</tr>
<tr>
<td>Amino acids w/o methionine</td>
<td>82.9 ± 8.7</td>
<td>100%</td>
</tr>
<tr>
<td>Methionine</td>
<td>133.7 ± 5.5</td>
<td>0%</td>
</tr>
</tbody>
</table>

Coelho et al., 1989

Environmental Conditions During the Preimplantation Period Can Impact the Offspring

Environmental Conditions
- Maternal undernutrition
- Maternal overnutrition
- Maternal obesity
- Maternal illness
- Assisted reproductive technologies

Preimplantation Period
- Trophoblaster
- Primate cytotrophoblast
- Syncytiotrophoblast

Potential Postnatal Outcomes
- Altered weight gain/adjustment
- Retarded growth and development
- Impaired glucose metabolism
- Altered behavior
- Modified immune response system
- Altered organs/size

Fleming et al., 2015
Effect of L-methionine Concentration on Blastocyst Development

Nutritional Effects from Pre-fresh to Early Pregnancy on Embryo Development and Fertility

It is now evident that nutritional effects on oocyte quality can originate when ovarian follicles emerge from the primordial pool and become committed to growth (approx. three to four months in cows). Undernutrition at this time reduces the number of follicles that emerge and therefore the number available to ovulate. Ashworth et al. 2009

Calving → Methionine enriched diets impact on embryo quality? → Improved Fertility?

The Growth of the Follicle Starts Prior to Calving

Calving = d 0, VWP = d 20.4, AI = d 30 ± 12 h

Follicular growth 100 days
Effects of Rumen-Protected Methionine or Choline Supplementation on the First Dominant Follicle

- 72 Holstein cows entering 2nd or greater lactation
- Experimental design was a randomized block design
- Housed in tie stalls with sand bedding
- Milked 3x per day
- Fed same basal TMR to meet but not exceed 100% of the energy requirements as outlined by NRC, 2001
  - From -34 d to calving: prepartum diet
  - From 0 to 30 DIM: fresh cow diet
  - From 31 to 72 DIM: high cow diet
- Treatments were given as top-dress

1. Rumen-protected methionine (MET; n = 20, received 0.08% of the DM of the diet/d as methionine, Smartamine M7, Adisseo, Alpharetta, GA, USA, to a Lys:Met = 2.9:1)
2. Rumen-protected choline (CHO; n = 17, received 60 g/d choline, Reassure, Balchem Corporation, New Hampton, NY)
3. Both rumen protected methionine and choline (MIX; n = 19, received 0.08% of the DM of the diet/d as methionine to a Lys:Met = 2.9:1 and 60 g/d choline)
4. No supplementation to serve as control (CON; n = 16, fed TMR with a Lys:Met = 3.5:1)

Blood Samples

Follicular Aspiration, 16mm (n=40)
Steroidogenesis Pathway

Serum AA Concentration from Cows at the Day of Follicular Aspiration of the Dominant Follicle of the 1st Follicular Wave Postpartum (~16 mm)

Follicular Fluid AA Concentration from Cows at the Day of Follicular Aspiration of the Dominant Follicle of the 1st Follicular Wave Postpartum (~16 mm)
Methionine Concentrations in Different Body Compartments of Dairy Cows

- Plasma: 16 to 35 µMol/L
- Uterine fluid: 31 to 46 µMol/L
- Oviduct: 31 to 49 µMol/L

Amino Acid Concentrations in the Oviduct and Uterus of Dairy Heifers

Synchronization Protocol
Effect of Methionine Supplementation from -21 DIM to 72 DIM on Lipid Accumulation of Preimplantation Embryos

Embryo development stages:
- Oocyte
- 2-cell
- 4-cell
- Morula
- Blastocyst

Fluorescence intensity of Nile Red staining

Effect of Methionine Supplementation from -21 DIM to 72 DIM on Lipid Accumulation of Preimplantation Embryos

Embryos (n=37) harvested 7 d after timed AI at 63 DIM from cows fed a control diet or the control diet enriched with methionine (Smartamine M).

Fluorescence intensity of Nile Red staining

P = 0.02
**DNA Methylation**

- Embryos up to 8 cell stage:
  - DNA is de-methylated

- 8 cell to Morula:
  - DNA is methylated
  - Modifies and add epigenetic information to the genome of the embryo. This process “epigenetic” is complete in the early embryonic stages.

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**Embryos exposed to embryokine CSF2 from day 5 to day 7 of development are different for the female and male embryo at day 15**

Embryos treated with CSF2 tended to be longer and produce more of the antiluteolytic signal IFNT at Day 15 of development than control embryos. 

Loureiro et al., Endocrinology 2010

Day 15 is an important point in time for the survival of the bovine embryo. At this time, the extraembryonic membranes (EEM) of the embryo are in the process of extensive elongation so that the embryo increases ~50-fold in length between Day 13 and Day 16.

Berg et al., Theriogenology 2010

CSF2: Colony-Stimulating Factor 2
Embryos exposed to embryokine CSF2 from day 5 to day 7 of development are different for the female and male embryo at day 15

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Effect of Supplementation with Smartamine M on Reproduction of Lactating Dairy Cows

- Holstein cows (n = 309, 138 primiparous, 171 multiparous)
- From 28 to 128 days in milk
- Cows were fed a basal TMR (6.9% Lys of MP and 1.87% Met of MP) and assigned to two treatments:
  - RPM: Basal TMR top dressed daily with Smartamine M
  - CON: Basal diet top dressed daily with DDG

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Effect of Supplementation with Smartamine M on Reproduction of Lactating Dairy Cows

RPM and CON cows fed the same TMR diet from 21 to 128 DIM
RPM cows were top dressed with 50 g (29 g DDG and 21 g of Smartamine M)
CON cows were top dressed with 50 g of DDG
### Animals

<table>
<thead>
<tr>
<th></th>
<th>CONTROL</th>
<th>RPM</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primiparous</td>
<td>68</td>
<td>70</td>
<td>138</td>
</tr>
<tr>
<td>Multiparous</td>
<td>85</td>
<td>86</td>
<td>171</td>
</tr>
<tr>
<td>TOTAL</td>
<td>153</td>
<td>156</td>
<td>309</td>
</tr>
</tbody>
</table>

### Production Data

<table>
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<tr>
<th>Item</th>
<th>CONTROL</th>
<th>RPM</th>
<th>P-value</th>
<th>RPM</th>
<th>P-value</th>
<th>RPM</th>
<th>P-value</th>
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<tbody>
<tr>
<td>Milk Test 1 (45-72 DIM)</td>
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<td></td>
<td></td>
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<tr>
<td>kg fat</td>
<td>23.4</td>
<td>38.4</td>
<td>0.02</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P-value</td>
<td>0.281</td>
<td>0.09</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>% fat</td>
<td>85.4</td>
<td>85.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>kg protein</td>
<td>2.36</td>
<td>2.36</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>P-value</td>
<td>0.25</td>
<td>0.25</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>kg fat (% milk)</td>
<td>33.6odel</td>
<td>33.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P-value</td>
<td>0.25</td>
<td>0.25</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>kg protein (% milk)</td>
<td>1.16</td>
<td>1.16</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>P-value</td>
<td>0.25</td>
<td>0.25</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Overall</td>
<td>23.6</td>
<td>42.1</td>
<td>0.0008</td>
<td></td>
<td></td>
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<td></td>
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### Plasma Methionine with 12 h After Top-dressing

<table>
<thead>
<tr>
<th>Item</th>
<th>Plasma methionine, nmol/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CONTROL</td>
</tr>
<tr>
<td>Primiparous</td>
<td>23.8</td>
</tr>
<tr>
<td>Multiparous</td>
<td>23.4</td>
</tr>
<tr>
<td>P-value</td>
<td>0.281</td>
</tr>
</tbody>
</table>

* 3 Primiparous and 1 multiparous pools each treatment;  
* 4 pools each treatment (4 cows/pool)
Conclusions

- Rumen-protected methionine increased methionine concentration in serum and follicular fluid of dairy cows.
- The cow’s pregnancy success starts during the transition phase.
- Amino acid balancing (methionine) from pre-fresh to confirmed pregnancy may not only improve milk production and composition, it may also improve embryo quality and reduce early embryo losses.