Why the renewed excitement about Ca status?

- High prevalence of subclinical hypocalcemia (SCH):
  - 25% of first lactation animals
  - 50% of second lactation and older cows
- High impact of SCH:
  - Immune competence
  - Metabolic health
  - Milk production
  - Reproductive performance

High Impact of SCH

- Blood Ca <8.6 mg/dL in first 3 DIM (Martinez et al., 2012):
  - Decreased neutrophil killing capacity
  - Increased risk of metritis and endometritis
  - Tendency for later pregnancy
- Blood Ca <8.4 mg/dL in first week (Chapinal et al., 2012):
  - Increased odds of DA
  - Decreased early lactation milk production
  - Reduction in pregnancy at 1st AI

Chapinal et al., 2013, Kimura et al., 2006, Martinez et al. 2013, Reinhardt et al., 2011
### Increasing Blood Calcium

- **PARATHYROID GLAND**
  - PTH secretion
  - PTH
  - Low blood calcium
  - Activated Vitamin D
  - Calcium excretion
  - Active Vitamin D
  - INTESTINE
  - Ca absorption
  - BONE
  - Release of Ca

*Goff et al., 2008*

### Metabolic Alkalosis

- **PARATHYROID GLAND**
  - PTH secretion
  - Calcium excretion
  - Low blood calcium
  - Activated Vitamin D
  - Ca absorption
  - INTESTINE
  - Release of Ca

*Goff et al., 2008, Goff et al., 2014*

### Altering Blood pH via Dietary Cation Anion Difference (DCAD)

- **Cations:** Sodium (+1), Potassium (+1)
- **Anions:** Chloride (-1), Sulfate (-2)
- More H⁺ in blood to maintain electroneutrality = Decreased pH
- Result:
  - Improved sensitivity of PTH receptor to PTH stimulation
  - Ca release from bone to offset pH drop (excreted from kidney until hypocalcemic condition occurs)

*Goff et al., 2014, Goff and Honst, 2003*

### Cows fed low DCAD have higher Ca and 1,25-(OH)₂ vitamin D after PTH administration

*Goff et al., 2014*
Strategies for application of DCAD for close-up dry cows

1) Low K ration + NO anion supplementation
   • Calculated DCAD ~ +10 mEq/100 g DM
   • Urine pH = 8.3 – 8.5

2) Low K ration + PARTIAL anion supplementation
   • Calculated DCAD ~ 0 mEq/100 g DM
   • Urine pH = 7 – 8

3) Low K ration + FULL anion supplementation
   • Calculated DCAD ~ -10 to -15 mEq/100 g DM
   • Urine pH = 5.5 – 6.0
   ***Necessary to regularly monitor urine pH and adjust ration

*DCAD in mEq/100 g DM = (Na + K) - (Cl + S)

Questions to answer about DCAD application:

• Can SCH be controlled with the use of a DCAD program?

• Are there benefits to partial anion supplementation?

• If anion supplementation is increased, are there increased benefits in mineral status and performance?

Objective

• Evaluate the effects of anion supplementation to cows during the prepartum period on:
  • periparturient mineral status
  • performance
  • metabolism
  • immune parameters
  • uterine health

Materials and Methods

• 30 multiparous cows per treatment
  • Completely randomized design
  • Restricted to balance for previous lactation milk production and lactation number

• 3 prepartum dietary treatments:
  - Low K control= +17.4 mEq/100 g diet DM
  - Medium DCAD= +3.7 mEq/100 g diet DM
  - Low DCAD= -10.5 mEq/100 g diet DM
  **Target urine pH= 5.5 – 6.0**
### Experimental Diet Ingredient Composition (kgs of DM/d)

<table>
<thead>
<tr>
<th>Ingredient (kgs DM/d)</th>
<th>Control</th>
<th>MedDCAD</th>
<th>LowDCAD</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMR Corn Silage</td>
<td>5.79</td>
<td>5.79</td>
<td>5.79</td>
</tr>
<tr>
<td>Wheat Straw</td>
<td>3.63</td>
<td>3.63</td>
<td>3.63</td>
</tr>
<tr>
<td>Amino Plus</td>
<td>1.04</td>
<td>1.04</td>
<td>1.04</td>
</tr>
<tr>
<td>Citrus Pulp</td>
<td>0.43</td>
<td>0.43</td>
<td>0.43</td>
</tr>
<tr>
<td>Soybean Hulls</td>
<td>0.30</td>
<td>0.30</td>
<td>0.30</td>
</tr>
<tr>
<td>Canola Meal</td>
<td>0.29</td>
<td>0.29</td>
<td>0.29</td>
</tr>
<tr>
<td>Molasses</td>
<td>0.09</td>
<td>0.09</td>
<td>0.09</td>
</tr>
<tr>
<td>Calcium Diphosphate</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
</tr>
<tr>
<td>Ground corn grain</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Salt</td>
<td>0.03</td>
<td>0.03</td>
<td>0.03</td>
</tr>
<tr>
<td>Vitamin Mix</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>Rumensin (mg)</td>
<td>318</td>
<td>318</td>
<td>318</td>
</tr>
<tr>
<td>Animate</td>
<td>-</td>
<td>0.25</td>
<td>0.52</td>
</tr>
<tr>
<td>Wheat Midds</td>
<td>0.42</td>
<td>0.34</td>
<td>0.25</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>0.37</td>
<td>0.36</td>
<td>0.35</td>
</tr>
<tr>
<td>Corn Distillers Ethanol</td>
<td>0.29</td>
<td>0.17</td>
<td>0.05</td>
</tr>
<tr>
<td>Magnesium Oxide</td>
<td>0.07</td>
<td>0.05</td>
<td>0.03</td>
</tr>
<tr>
<td>Urea</td>
<td>0.05</td>
<td>0.03</td>
<td>-</td>
</tr>
</tbody>
</table>

### Urine pH, Intake & Diets

- Urine sampling
  - pH determined 3X weekly prior to calving
  - Dietary adjustments made as needed
- Dry Matter Intake
  - Feed delivered and refused recorded daily
  - Fed with target of 10% refusals to allow ad libitum intake
- TMR and Diet Ingredients
  - Sampled weekly for dry matter determination
  - Composited over 4 weeks and analyzed by Cumberland Valley Analytical Services

### Production & Energy Status

- Milk production
  - Cows milked 3X per day
  - Daily milk weights recorded
  - Samples collected at all milkings on one day per week analyzed by midinfrared techniques for:
    - fat, true protein, lactose, total solids, urea-N, and somatic cell count (DairyOne Laboratories, Ithaca, NY)
- Body Weight and Body Condition Score
  - Recorded weekly
  - BCS averaged over two scorers (Wildman et al., 1982)
Blood Sampling Scheme

Once prior to treatment
Begin Treatment Diets
Twice weekly until calving
Twice in 24 hrs & daily through 5 days
1X weekly through 56 days
-31 -24 -21 -14 -7 0 7 14 21
Prepartum and postpartum data analyzed separately
- MIXED procedure of SAS 9.4 with the repeated statement
- Covariates values included in the model when available
  - Fixed effects = treatment, time, parity (2nd vs. 3rd+), and two way interactions
  - Effect of decreasing DCAD was tested using orthogonal contrasts
  - Differences in frequency of hypocalcemia determined using Fisher’s Exact Test
  - LSMEANS and standard errors reported throughout
  - Significance declared at P ≤ 0.05, trends discussed at 0.05<P≤0.10

Analyzed Diet Composition (Mean ± S. D.)

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Control (Mean ± S.D.)</th>
<th>MedDCAD (Mean ± S.D.)</th>
<th>LowDCAD (Mean ± S.D.)</th>
<th>Lactating (Mean ± S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM (% DM)</td>
<td>46.3 ± 1.8</td>
<td>46.5 ± 1.3</td>
<td>46.4 ± 1.1</td>
<td>45.7 ± 1.8</td>
</tr>
<tr>
<td>CP (% DM)</td>
<td>13.0 ± 0.3</td>
<td>13.2 ± 0.4</td>
<td>13.2 ± 0.5</td>
<td>15.7 ± 0.2</td>
</tr>
<tr>
<td>ADF (% DM)</td>
<td>30.2 ± 0.7</td>
<td>30.5 ± 1.3</td>
<td>30.1 ± 1.3</td>
<td>20.6 ± 0.8</td>
</tr>
<tr>
<td>NDF (% DM)</td>
<td>44.3 ± 1.2</td>
<td>44.0 ± 2.1</td>
<td>43.2 ± 1.8</td>
<td>31.1 ± 1.0</td>
</tr>
<tr>
<td>Starch (% DM)</td>
<td>17.0 ± 0.5</td>
<td>16.3 ± 0.8</td>
<td>16.3 ± 0.9</td>
<td>26.0 ± 0.7</td>
</tr>
<tr>
<td>NFC (% DM)</td>
<td>13.4 ± 0.9</td>
<td>34.3 ± 2.5</td>
<td>35.0 ± 1.9</td>
<td>45.8 ± 1.2</td>
</tr>
<tr>
<td>Fat (% DM)</td>
<td>1.1 ± 0.1</td>
<td>1.3 ± 0.2</td>
<td>1.1 ± 0.3</td>
<td>2.3 ± 0.2</td>
</tr>
<tr>
<td>Ca (% DM)</td>
<td>1.54 ± 0.12</td>
<td>1.57 ± 0.14</td>
<td>1.57 ± 0.07</td>
<td>0.95 ± 0.03</td>
</tr>
<tr>
<td>P (% DM)</td>
<td>0.44 ± 0.01</td>
<td>0.43 ± 0.01</td>
<td>0.41 ± 0.01</td>
<td>0.41 ± 0.02</td>
</tr>
<tr>
<td>Mg (% DM)</td>
<td>0.47 ± 0.01</td>
<td>0.48 ± 0.01</td>
<td>0.50 ± 0.03</td>
<td>0.44 ± 0.02</td>
</tr>
<tr>
<td>K (% DM)</td>
<td>1.28 ± 0.07</td>
<td>1.26 ± 0.06</td>
<td>1.24 ± 0.07</td>
<td>1.37 ± 0.05</td>
</tr>
<tr>
<td>S (% DM)</td>
<td>0.20 ± 0.01</td>
<td>0.30 ± 0.02</td>
<td>0.41 ± 0.02</td>
<td>0.29 ± 0.01</td>
</tr>
<tr>
<td>Fe (% DM)</td>
<td>0.33 ± 0.01</td>
<td>0.33 ± 0.01</td>
<td>0.34 ± 0.01</td>
<td>0.44 ± 0.02</td>
</tr>
<tr>
<td>Cu (% DM)</td>
<td>0.27 ± 0.03</td>
<td>0.47 ± 0.05</td>
<td>0.69 ± 0.04</td>
<td>0.40 ± 0.02</td>
</tr>
<tr>
<td>DCAD (mEq/100g DM)</td>
<td>18.3 ± 0.8</td>
<td>5.9 ± 3.4</td>
<td>7.4 ± 3.6</td>
<td>25.0 ± 1.5</td>
</tr>
</tbody>
</table>

Statistical Analysis

Urine pH

<table>
<thead>
<tr>
<th>Prepartum Diet</th>
<th>CON (Mean ± S.D.)</th>
<th>MedDCAD (Mean ± S.D.)</th>
<th>LowDCAD (Mean ± S.D.)</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine pH</td>
<td>8.20 (8.07-8.32)</td>
<td>7.84 (7.72-7.96)</td>
<td>5.98 (5.87-6.10)</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Urine pH

Day Relative to Calving

Prepartum Plasma Minerals

<table>
<thead>
<tr>
<th>Variable</th>
<th>CON</th>
<th>MedDCAD</th>
<th>LowDCAD</th>
<th>SEM</th>
<th>Linear Contrast</th>
<th>Quadratic Contrast</th>
<th>Trt×Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>9.45</td>
<td>9.58</td>
<td>9.59</td>
<td>0.09</td>
<td>0.34</td>
<td>0.54</td>
<td>0.64</td>
</tr>
<tr>
<td>Magnesium</td>
<td>1.84</td>
<td>1.85</td>
<td>1.79</td>
<td>0.03</td>
<td>0.17</td>
<td>0.39</td>
<td>0.13</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>6.16</td>
<td>6.05</td>
<td>6.11</td>
<td>0.10</td>
<td>0.71</td>
<td>0.52</td>
<td>0.77</td>
</tr>
</tbody>
</table>

Postpartum (d 0-14) Plasma Minerals

<table>
<thead>
<tr>
<th>Variable</th>
<th>Prepartum Diet</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Prepartum Diet</td>
<td>P-values</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>MedDCAD</td>
</tr>
<tr>
<td>P-values</td>
<td>Linear Contrast</td>
<td>Quadratic Contrast</td>
</tr>
<tr>
<td>Calcium</td>
<td>8.84</td>
<td>8.89</td>
</tr>
<tr>
<td>Magnesium</td>
<td>1.72</td>
<td>1.76</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>4.74</td>
<td>4.67</td>
</tr>
</tbody>
</table>

Plasma Calcium

Day Relative to Calving

**Plasma Magnesium**

- Prepartum: Trt P=0.28, Day P=0.02, Trt×Day P=0.13
- Postpartum: Trt P=0.35, Day P<.0001, Trt×Day P=0.008


**Postpartum Plasma Calcium Treatment by Parity Interaction**

- 2nd Parity: Control, MedDCAD, LowDCAD
- 3rd+ Parity: Trt x Parity P=0.06


**Hypocalcemia (Ca<8.5 mg/dL): 2nd Lactation**

- Percent
- Day Relative to Calving


**Hypocalcemia (Ca<8.5 mg/dL): 3rd+ Lactation**

- Percent
- Day Relative to Calving

Dry Matter Intake

<table>
<thead>
<tr>
<th>Variable</th>
<th>Prepartum Diet</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (CON)</td>
<td>MedDCAD</td>
</tr>
<tr>
<td>Prepartum DMI, kg/d</td>
<td>14.55</td>
<td>15.08</td>
</tr>
<tr>
<td>Prepartum DMI, % of BW</td>
<td>1.87</td>
<td>1.89</td>
</tr>
<tr>
<td>Postpartum (wk 1 to 3) DMI, kg/d</td>
<td>20.99</td>
<td>21.74</td>
</tr>
<tr>
<td>Postpartum (wk 1 to 9) DMI, kg/d</td>
<td>24.73</td>
<td>25.67</td>
</tr>
</tbody>
</table>

Energy Balance: Through Week 9

Milk Production: Weeks 1 to 3

<table>
<thead>
<tr>
<th>Variable</th>
<th>Prepartum Diet</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (CON)</td>
<td>MedDCAD</td>
</tr>
<tr>
<td>Milk yield, kg/d</td>
<td>40.54</td>
<td>42.13</td>
</tr>
<tr>
<td>Fat, %</td>
<td>4.38</td>
<td>4.38</td>
</tr>
<tr>
<td>Fat, kg</td>
<td>1.74</td>
<td>1.81</td>
</tr>
<tr>
<td>3.5% FCM, kg/d</td>
<td>45.63</td>
<td>47.52</td>
</tr>
<tr>
<td>True protein, %</td>
<td>3.54</td>
<td>4.19</td>
</tr>
<tr>
<td>True protein, kg</td>
<td>1.36</td>
<td>1.42</td>
</tr>
<tr>
<td>Lactose, %</td>
<td>4.64</td>
<td>4.67</td>
</tr>
<tr>
<td>Lactose, kg</td>
<td>1.89</td>
<td>1.98</td>
</tr>
<tr>
<td>Total Solids, %</td>
<td>13.63</td>
<td>13.61</td>
</tr>
<tr>
<td>Total Solids, kg</td>
<td>5.42</td>
<td>5.65</td>
</tr>
<tr>
<td>ECM, kg/d</td>
<td>46.12</td>
<td>48.04</td>
</tr>
<tr>
<td>MUN, mg/dL</td>
<td>10.32</td>
<td>9.72</td>
</tr>
<tr>
<td>SCS</td>
<td>2.62</td>
<td>3.26</td>
</tr>
</tbody>
</table>
Impact of Decreasing DCAD in the Prepartum Diet

- Mineral Status
  - Higher plasma Ca for several days postpartum
  - Decreased hypocalcemia incidence in older cows
  - Lower postpartum Mg – what are the implications?

- Early Lactation Performance (Weeks 1 to 3):
  - Increased postpartum DMI
  - Increased milk yield
  - Increased fat corrected milk yield (trend)
  - Increased energy-corrected milk yield (trend)

Questions to answer about DCAD application:

- Can SCH be controlled with the use of a DCAD program?
  - Improved Ca status overall, lower SCH incidence in older cows

- Are there benefits to partial anion supplementation?
  - Mixed response of MedDCAD group, but linear responses in blood calcium, intake and production indicates there is some benefit

- If anion supplementation is increased, are there increased benefits in mineral status and performance?
  - Greatest blood calcium, intake and production responses in LowDCAD group
# Acknowledgements

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Dani Harris  
Jaco Webb  
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