New Insights into Calcium Intake in Transition Dairy Cows
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Change in Calcium Status at Parturition

- During **late pregnancy** calcium is lost from the blood to the fetus at a rate of 80 mg/kg$^{3/4}$ (Horst et al., 2006)
- During **early lactation** calcium is lost from the blood to the milk at a rate of 500 mg/kg$^{3/4}$ (Horst et al., 2006)
- Cows will deplete their blood pool of calcium 20-30x per day in order to put sufficient calcium into milk (Goff, 2008)
- Right at calving, bone resorption is decreased in dairy cattle (Horst et al., 2006)
- Lose calcium much more quickly than other species due to complete milk removal at 2-3x/day

Kovacs and Kronenberg, 1997
Calcium loss associated with pregnancy and lactation

Growing Fetus $\rightarrow$ 3-6 g/d
Colostrum $\rightarrow$ ~ 23 g/d
Early Milk $\rightarrow$ 30-50 g/d
Peak Milk $\rightarrow$ ~ 80 g/d

Hypocalcemia

- **Incidence**: 5-10% clinical (<1.4 mM), 25-50% subclinical (1.4-2.0 mM)
- **Implication**: Cow loss, down cows, other health problems
- **Cost**: $150-$300 per case (for treatment/lost MY)
  - 9.2 million cows
  - ~$900,000,000
  - 1.27 million cows in WI
  - Total cost ~$125,000,000
  - Annual cost to average WI farm ~$12,000
Hypocalcemia

Smooth muscle function
- Rumen and GIT motility
- Feed intake
- Milk yield
- Energy balance
- Ketosis
- Fatty liver

Immune function
- Uterine motility
- Dystocia (RFM)
- Teat sphincter contraction
- Mastitis

- Reduced pregnancy rates
- Longer intervals to pregnancy (Martinez et al., 2012)
- Production losses ~14% (Guard, 1996)

Calcium Regulation During Lactation

NORMAL
- Calcium Intake
- Intestinal Absorption
- Serum Ca^{2+}
- Skeletal Calcium
- Renal Calcium
- Urine

PREGNANCY
- Calcium Intake
- Intestinal Absorption
- Serum Ca^{2+}
- Skeletal Calcium
- Renal Calcium
- Placental Calcium Pump
- Fetus

LACTATION
- Calcium Intake
- Intestinal Absorption
- Serum Ca^{2+}
- Skeletal Calcium
- Renal Calcium
- Breast Milk
- Neonate

Goff, 2008; Martín-Tereso and Verstegen, 2011; Chapinal et al., 2011

Kovacs and Kronenberg, 1997
Prevention or Treatment?

**DCAD (Pre-partum diet)**
- (mEq K⁺ + mEq Na⁺) – (mEq Cl⁻ + mEq 0.6 S0₄²⁻)
- Anionic salt programs
- Induction of metabolic acidosis
- To date, most successful strategy
  - -15mEq/100g for hypocalcemia prevention

**Low Calcium Diets (Pre-partum)**
- Efficacy close to 100% when daily Ca is below 20g/d (Thilsing-Hansen et al., 2002)
- Difficult to formulate dry cow ration with < 1.5g dietary Ca per kg DM

Strategies for Prevention -Postpartum

**Oral Calcium (primarily Calcium Chloride)**
- Ca bolus given prophylactically helped maintain blood Ca (Oetzel, 1996)
- Less of an impact seen with introduction of DCAD diets (Oetzel and Miller, 2012)
  - Beneficial to treat lame and high producing ≥ 2nd lactation cows
    - ↑ 1st test milk by 3.1 kg
    - ↓ adverse health events
Risk of Milk Fever Based on DCAD Balance

Fig. 8. Equations describing the relation between DCAD and daily urinary Ca excretion (equation from Roche and colleagues\(^{22}\)) and milk fever incidence (equation from Charbonneau and colleagues\(^{129}\)). (Data from Refs. 20, 20.129)

Martin-Tero and Martens, 2014

Effects of Ca Supplementation during Dry Period

Figure 4. Sample relationship of dietary Ca to the incidence of milk fever using the final regression model. Points plotted were calculated using the mixed breed intercept, lactation number = 5, Na = .20%, and S = .35%.

Oetzel, 1991; Lean et al., 2006
**The Importance of Ca\(^{2+}\)**

- **Key mineral in milk**
  - Largely bound to proteins and anionic carriers (phosphate, citrate, bicarbonate)

- **Critical intracellular signaling molecule**
  - Cannot be synthesized or destroyed so must be controlled by compartmentalization and buffering inside cells

- **Master regulator of systemic Ca\(^{2+}\) is the CALCIUM SENSING RECEPTOR (CaSR)----a G protein coupled receptor**
  - Controlled by PTH and PTHrP

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**Mammary Epithelial Cell Ca\(^{2+}\) Dynamics**

40% exported this way

60% exported this way

Neville, 2005; Shennan, 2008; Cross et al., 2014
Calcium Flux During Lactation

- Active Transport
- Passive Transport

Dietary Ca: 45-150 g
Extracellular Ca Pool: 11 g

3-3.5 g Ca^{2+}

20-30 g

7.8-8.5 kg

9-13% Bone Mass lost during 1st 30 DIM

Colostrum Ca^{2+}: 1.7 g-2.3 g/kg
Milk Ca^{2+}: 1.1 g/kg

How are the MG and bone systems inter-related?
What are the systemic factors / signals?

Calcium mobilization from bone is stimulated by mammary secretion of parathyroid hormone-related protein (PTHrP)

- Closely related at its N-terminal end to PTH
- Signals through the same GPCR
- Synthesized and secreted by “non-parathyroid gland tissues”
  - Paracrine/autocrine factor
- Only detected in the circulation during lactation and during metastatic bone cancer
Ca movement in the mammary gland controls systemic Ca concentrations

Serotonin Synthesis

Adapted from Wang et al., 2002
**PTHrP is decreased in the mammary gland and circulation of TPH1 deficient mice**

**Rat Feeding Study**

5-HTP fed dams were able increase circulating serum and milk Ca$^{2+}$ concentrations, or at least maintain them comparable to the CON dams

Hernandez et al., 2012; AJP

Laporta et al., 2013; DAE
Tph1 Deficient Mouse Study

Femurs were collected on day 10 of lactation


What Does this Have to Do with Calcium in Dairy Cows?
Relationship of Serotonin and Calcium on D1 Lactation in Dairy Cattle

Laporta et al., 2013: JDS

Farm 1-Jerseys

Moore et al., 2015; JDS
Can We Give Dairy Cows 5-HTP and Impact Calcium Metabolism?

Moore et al., 2015; JDS
Can We Give Dairy Cows 5-HTP and Impact Calcium Metabolism Post-Calving? Are there differences between how Holstein and Jersey Cows respond?
5-HTP infusion pre-calving improves Ca at Transition

Weaver et al., 2016: J. Endocrinol.

5-HTP infusion pre-calving increases post-partum Ca in Swiss Cows

Hernandez-Castellano et al., 2017; JDS
Serotonin interacts with DCAD

Theory of How DCAD Works

Goff, 2008
Negative DCAD diet pre-calving increases serotonin

Martinez et al., unpublished

Combination of a negative DCAD diet with supplemental 5-HTP pre-partum results in elevated IONIZED calcium post-partum

Slater et al., In Review
Combination of a negative DCAD diet with supplemental 5-HTP prepartum results in elevated TOTAL calcium post-partum

Serotonin Concentrations During the Prepartum Period
Cart or Horse? Serotonin or Calcium?

Total Blood Ca: 2.2 mM—2.6 mM

Blood Ca\(^{2+}\) → ORAI1 → PMCA2 → Serotonin

Blood Ca\(^{2+}\) → ORAI1 → PMCA2 → Serotonin

Milk Total Ca (123mg/100 ml of milk or 3.08 mM; ~50 g in 100 pounds of milk)

Dietary Treatments

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<th>LOW</th>
<th>MEDIUM</th>
<th>HIGH</th>
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<td>Target</td>
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<tr>
<td>Cl</td>
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<tr>
<td>DCAD</td>
<td>-15.1</td>
<td>-16.7</td>
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*Final Dietary Period Began on June 5 and first infusions started on June 27 ending June 29 (6 cows per day; 2 per treatment)

\(\text{DMI} = 12.7 \text{ kg for all cows and no feed was left behind}\)

N=5  N=6  N=6

57.2 g Calcium  143.5 g Calcium  256.6 g Calcium
Experimental Timelines

21 D Dietary Feeding Period
- D 1
- D 19: Catheters Inserted
- D 22, D 23, D 24: EGTA Challenges

Induction Period-5% EGTA (500ml/h)
- Start
- 15 min
- Every 15 min
- 60% of initial blood ionized calcium achieved

Recovery Period
- 0, 2.5, 5, 10, 15, 30
- Every 30 min
- 90% of initial blood ionized calcium achieved

Rumination Minutes-SCR Collars

- Low Calcium
- Medium Calcium
- High Calcium

rumination minutes

Trt: P = 0.24
Time: P < 0.001
Trt*Time: P = 0.21

Amundson et al., unpublished
Average Urine pH over 21 day feeding period

- **Trt**: $P = 0.02$
- **Time**: $P = 0.20$
- **Trt*Time**: $P = 0.32$

Ionized Calcium-5% EGTA Challenge Period

- **Trt**: $P = 0.03$
- **Time**: $P < 0.0001$
- **Trt*Time**: $P = 0.38$
Ionized Calcium-5% EGTA Recovery Period

Amundson et al., unpublished

Average time to 60% of ionized Calcium during 5% EGTA induction

Amundson et al., unpublished
Total G EGTA infused to reach 60%

- **Trt**: $P = 0.01$

Response of serotonin to calcium chelation

- **Trt**: $P = 0.05$
- **Time**: $P = 0.60$
- **Trt*Time**: $P = 0.78$

Amundson et al., unpublished
Serotonin and Calcium regulate calcium movement in the gland in order to stimulate bone resorption.