How can we collect and run blood samples for calcium determination?

Jessica A. A. McArt, DVM, PhD, DABVP (Dairy Practice)

Population Medicine & Diagnostic Sciences
College of Veterinary Medicine
Cornell University
Ithaca NY 14853

Overview

- Determining which cows are dyscalcemic
- Direct measurement of calcium
- On-farm blood testing recommendations
- Indirect measurements of calcium

Direct measurement of calcium

- Calcium is found in 3 forms in blood:
  - Free or ionized (50-60%)
  - Bound to proteins (30%)
  - Complexed (10%)

- 2 options:
  - Ionized calcium (iCa)
  - Total calcium (tCa)

Ionized calcium

- iCa thought to have greater biological relevance than tCa
- Ion-selective electrode technology is largely employed for clinical use (blood-gas analyzers)
- Measurement of iCa is expensive, special handling procedures
  - Heparin salts bind calcium
  - Use of electrolyte-balanced syringes
  - Exposure to air changes blood pH

Ionized calcium – methods of analysis

- Cowside = not practical

- Machines targeted for on-farm use:
  - iSTAT, VetScan, Nova Stat
  - $15,000-$20,000 + sample costs

- Fast, accurate, and <u>inexpensive</u> tools that measure iCa do not currently exist

Total calcium (tCa)

- Can collect in:
  - Red top tubes (non-anticoagulant; serum)
  - Green top tubes (heparin; plasma)
- Should not collect in:
  - Purple top tubes (EDTA; plasma)
  - Binds calcium so will get a very low tCa results
- Methods of analysis:
  - Benchtop analyzer in laboratory @ US$10-20/sample

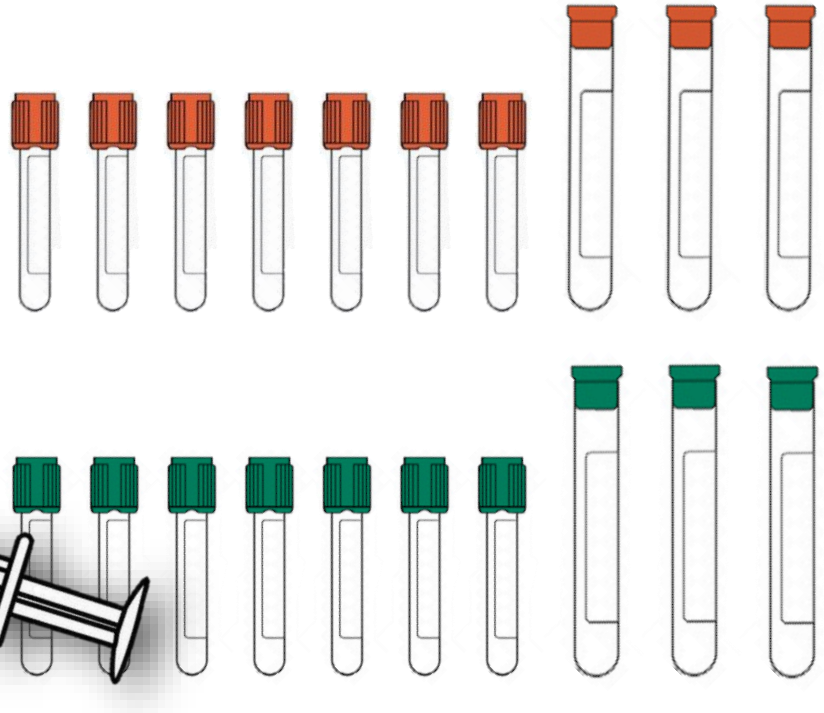
Total calcium – how stable is it?

July 2017 to April 2018
2 New York dairies

30

~30%
≤2.1 mmol/L

19



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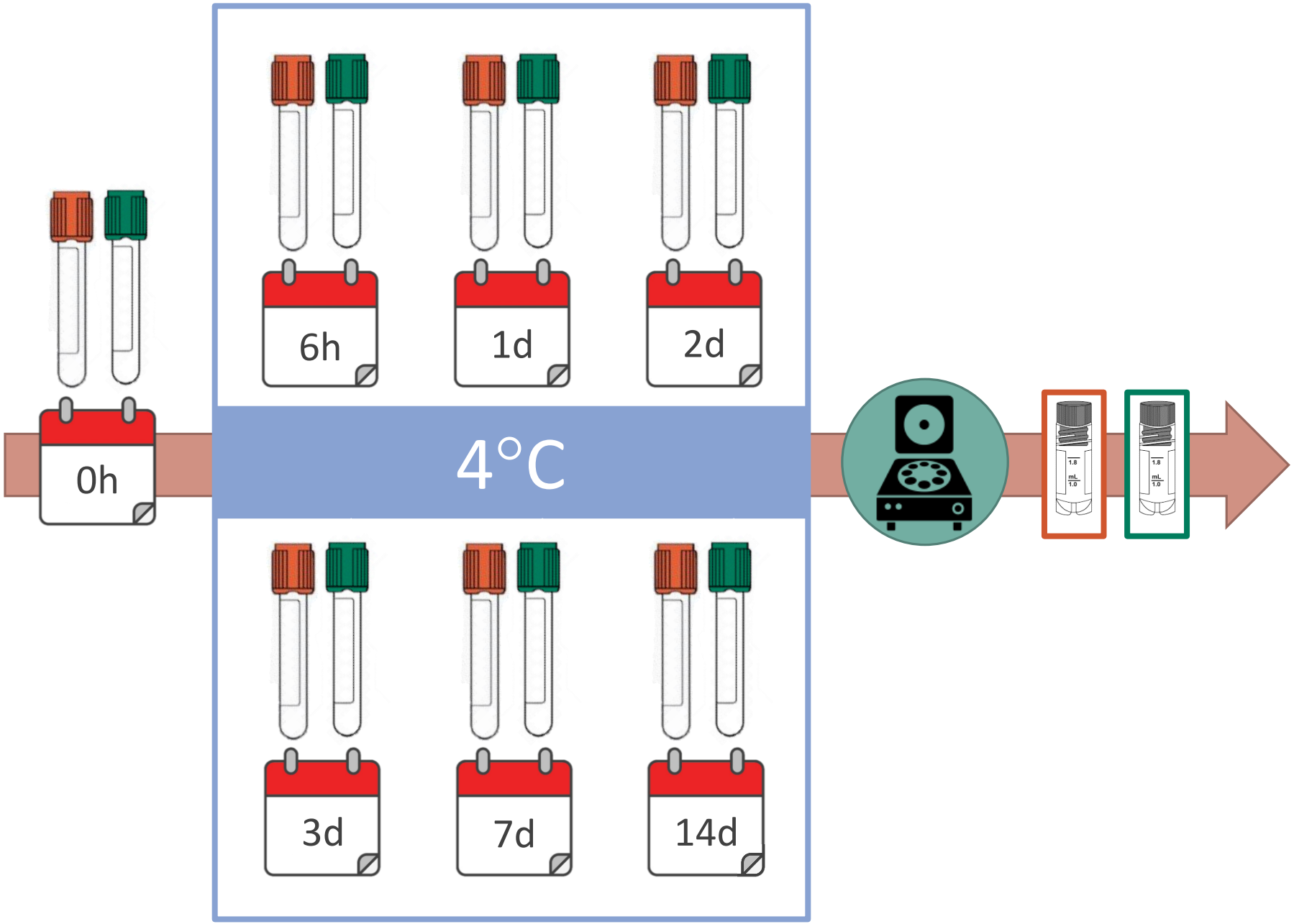
Technical note: **Effect of storage time and temperature on total calcium concentrations in bovine blood**

K. D. Bach,[1] R. C. Neves,[2] T. Stokol,[1] and J. A. A. McArt[1]*
[1]Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853
[2]Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Purdue University, West Lafayette, IN 47907

Adapted from K. D. Bach



Animal Health Diagnostic Center

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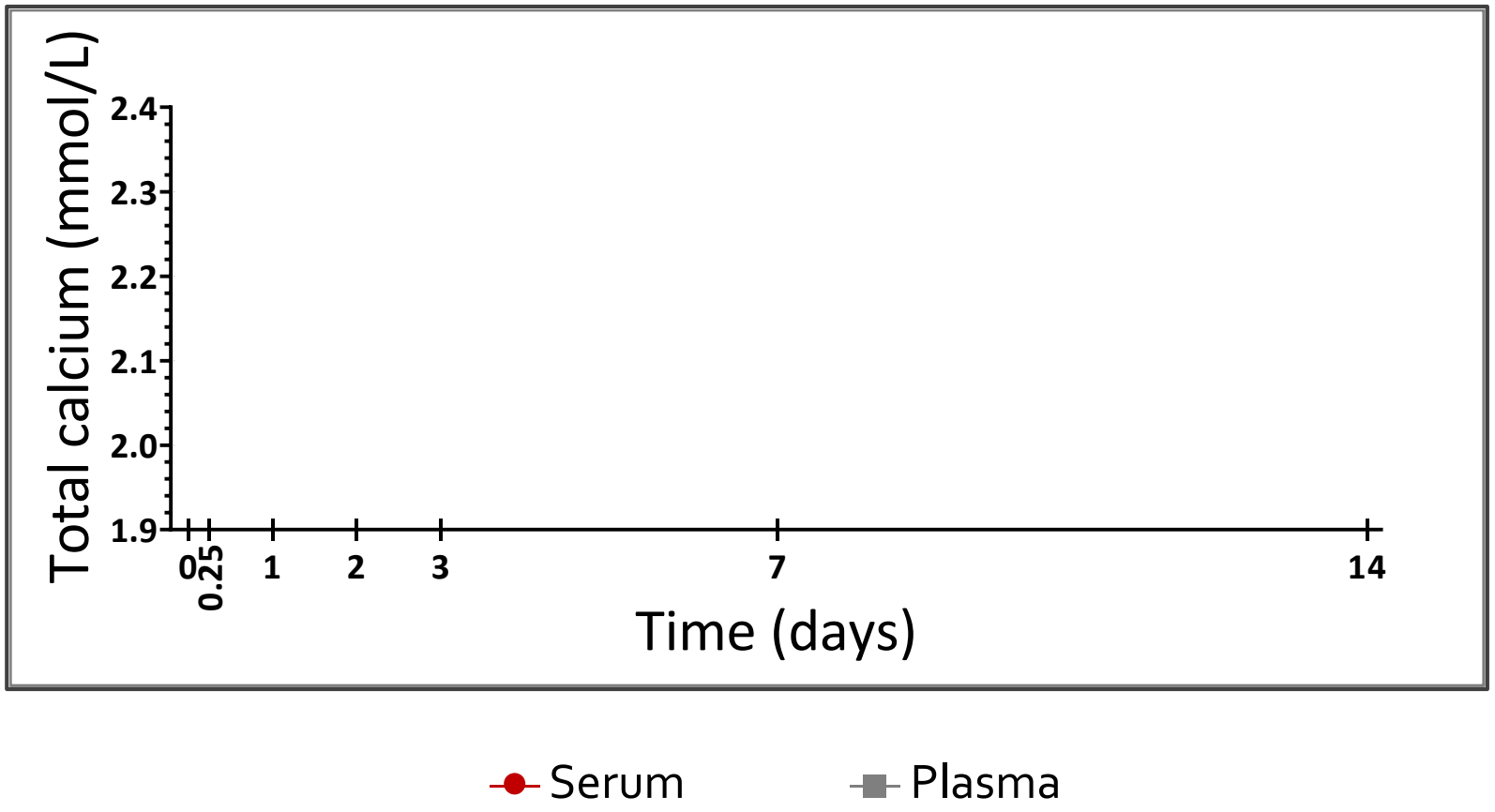
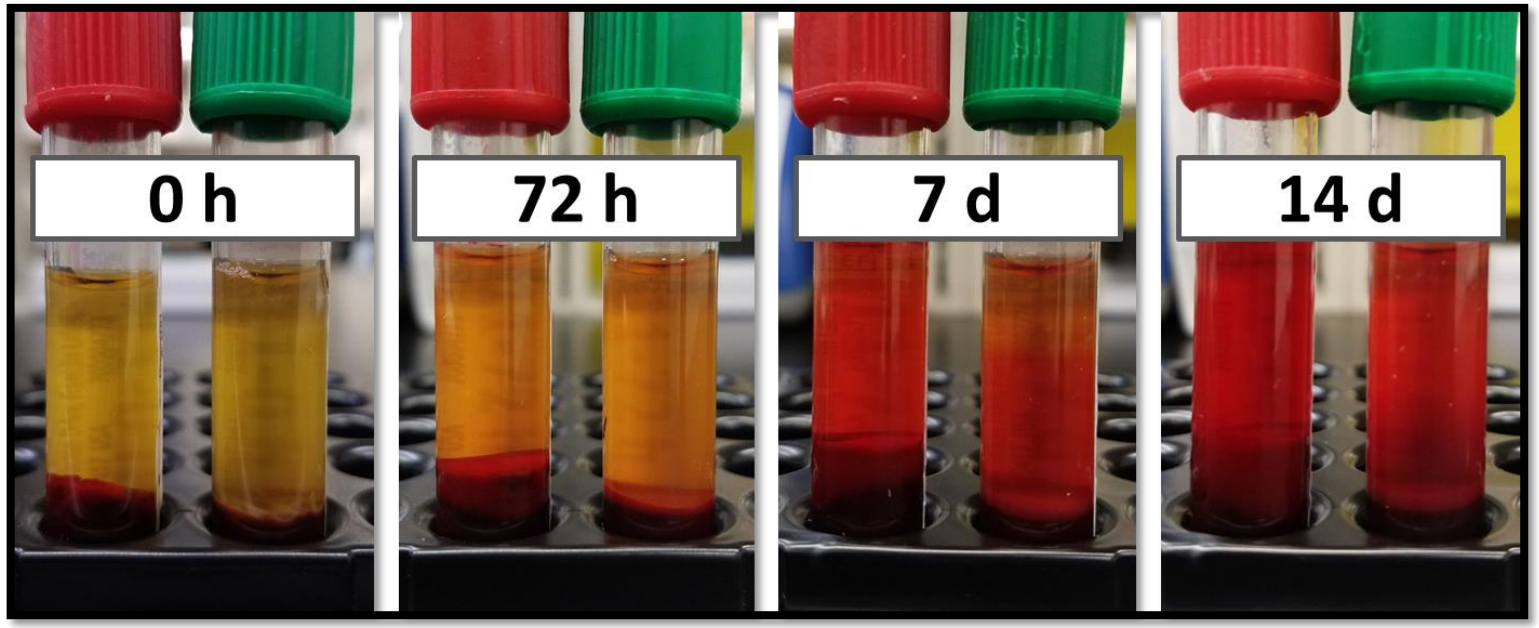
Whole blood stored at 4°C



- No difference between serum & plasma ($P = 0.4$)
- No change in tCa versus 0 hr (all $P > 0.1$)

Adapted from K. D. Bach

Effect of hemolysis?



- No effect on tCa (P = 0.03)
- Not true for NEFA (Stokol & Nydam, 2006)

	Hemolysis, units median (range)			
Serum	7 (3 to 18)	31 (20 to 51)	99 (30 to 241)	351 (58 to 1,687)
Plasma	6 (2 to 14)	32 (15 to 76)	83 (44 to 234)	169 (64 to 1,058)

Dunnett's: all $P < 0.001$

Adapted from K. D. Bach

How should we use on-farm testing?

- No current practical, on-farm testing methods
  - Exception: iStat type units
  - Exception: farms willing to purchase benchtop units

- *Milk fever:* collect blood from down cows before treatment
  - Store in a working fridge!
  - Save and test if no response to treatment

- *Routine dyscalcemia monitoring:* take blood from cows at 4 d in milk
  - Store in a working fridge!
  - Submit to lab all at once after appropriate sample size

Can we estimate calcium indirectly?

Indirect measurement of calcium

- Reduces need to lock up cows
- Provides immediate information

- Historical method: cold ears
- Rumination and activity time
- Proportional milk analysis

Cold ears?

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Evaluation of ear skin temperature as a cow-side test to predict postpartum calcium status in dairy cows

P. L. Venjakob,*† S. Borchardt,* G. Thiele,† and W. Heuwieser*[1]
*Clinic for Animal Reproduction, Faculty of Veterinary Medicine, Freie Universität Berlin, Koenigsweg 65, 14163 Berlin, Germany
†Veterinary practice G. Thiele, Baruth, Germany

- 7 herds
- 251 cows, 0-48 hr postpartum

- Manual scoring
- Rectal temperature
- Infrared thermometer
- Blood calcium

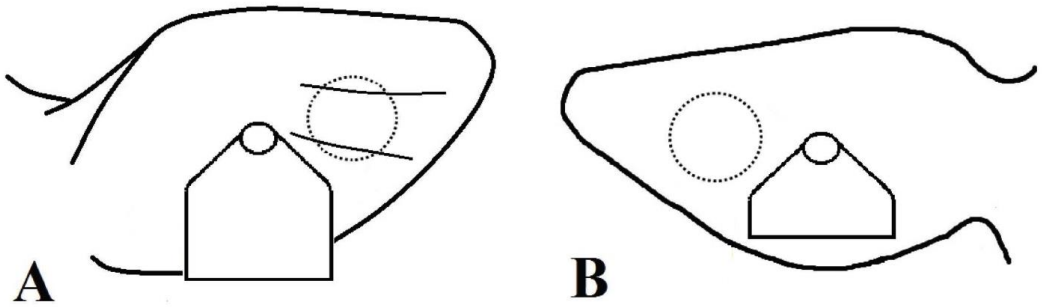


Figure 1. Schematic presentation of the measuring points for the infrared thermometer on the front (A) and rear side (B) of the ear.

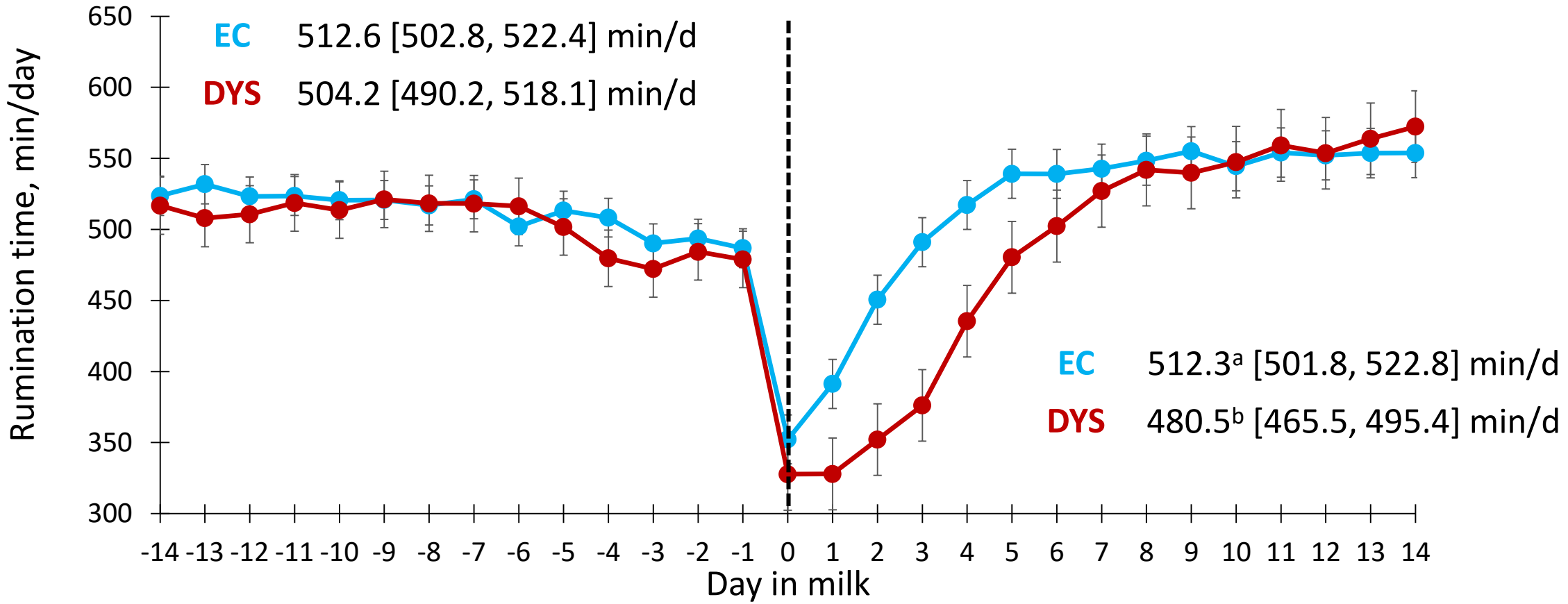
- Hypocalcemia defined as blood calcium < 2.0 mmol/L

Calcium threshold, mmol/L	Prevalence, %	Temperature variable[1]	Threshold, °C	Sensitivity	Specificity	AUC[2]	*P*-value
2.0	29.6	STEar	27.0	49.3	73.8	0.641	0.001
		STCox	30.0	52.2	78.7	0.668	0.001
		RT	39.0	75.4	42.7	0.606	0.009

- Decrease in ear temp of 0.39°C associated with decrease of 0.1 mmol/L in calcium
- Ambient temp was a major confounder
- Conclusions: ear temperature cannot be recommended for diagnosis of subclinical hypocalcemia

Rumination time is different in dyscalcemic cows

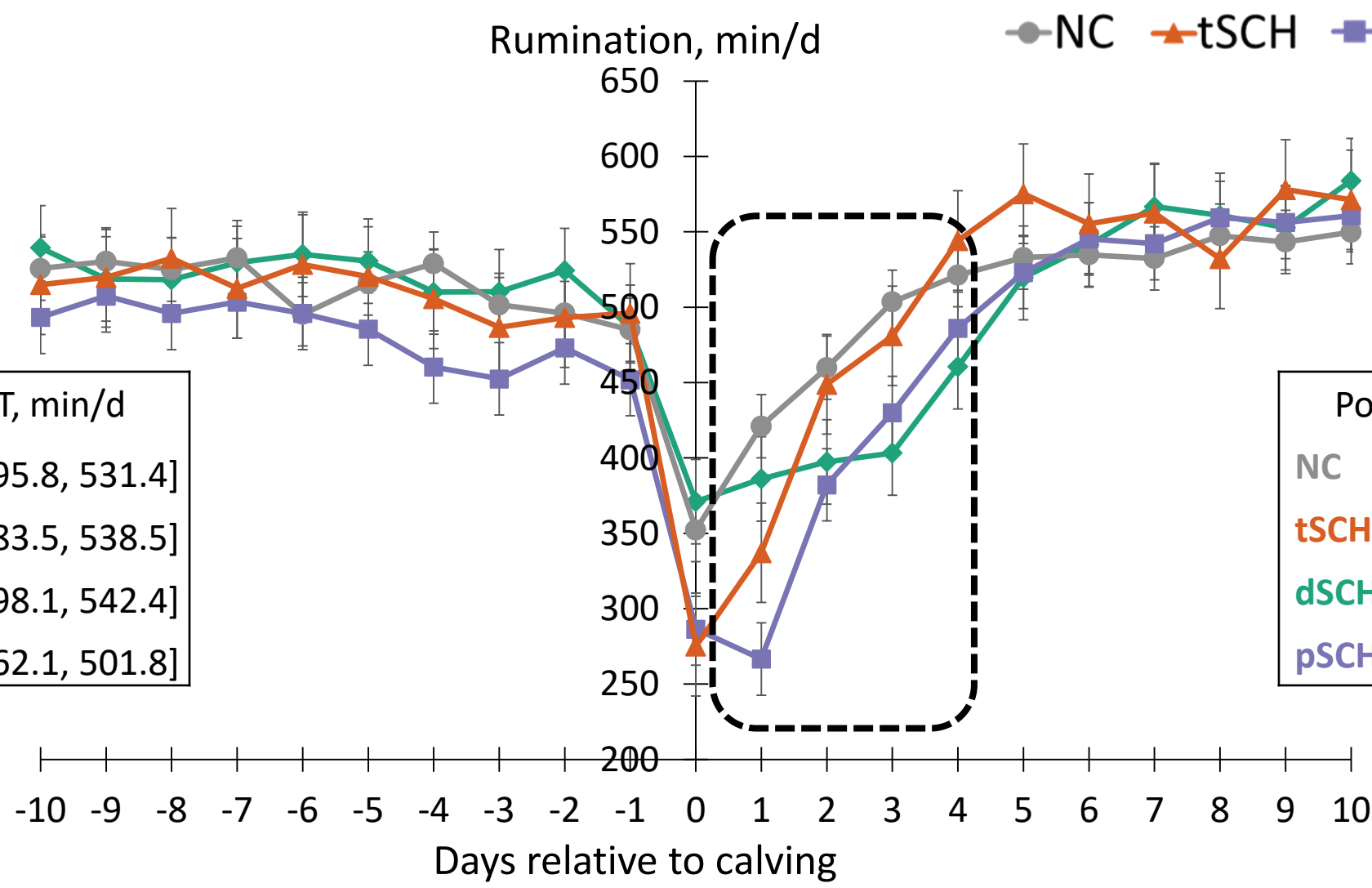
Eucalcemic (EC; n = 125): tCa > 2.2 mmol/L at 4 DIM
Dyscalcemic (DYS; n = 57): tCa ≤ 2.2 mmol/L at 4 DIM



Error bars = 95% CI

Courtesy: C. Seely

Rumination time & calcium dynamics



Prepartum RT, min/d	
NC	513.6 [495.8, 531.4]
tSCH	511.0 [483.5, 538.5]
dSCH	520.3 [498.1, 542.4]
pSCH	481.9 [462.1, 501.8]

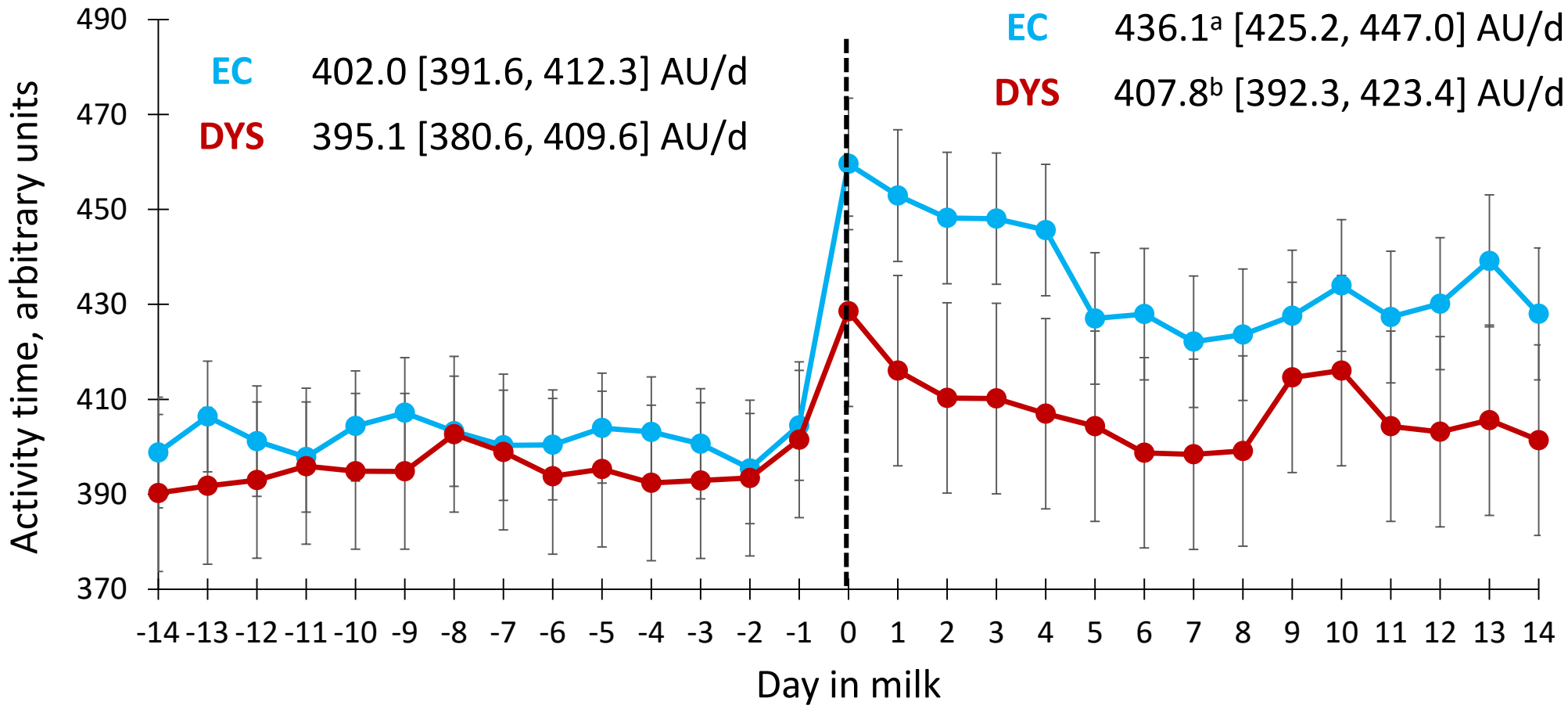
Postpartum RT, min/d	
NC	499.8 [478.2, 521.4]
tSCH	496.4 [462.9, 530.0]
dSCH	485.8 [457.5, 514.1]
pSCH	467.1 [443.0, 491.3]

*error bars represent 95% CI

Seely & McArt; unpublished data

Activity time is different in dyscalcemic cows

Eucalcemic (EC; n = 125): tCa > 2.2 mmol/L at 4 DIM
Dyscalcemic (DYS; n = 57): tCa ≤ 2.2 mmol/L at 4 DIM



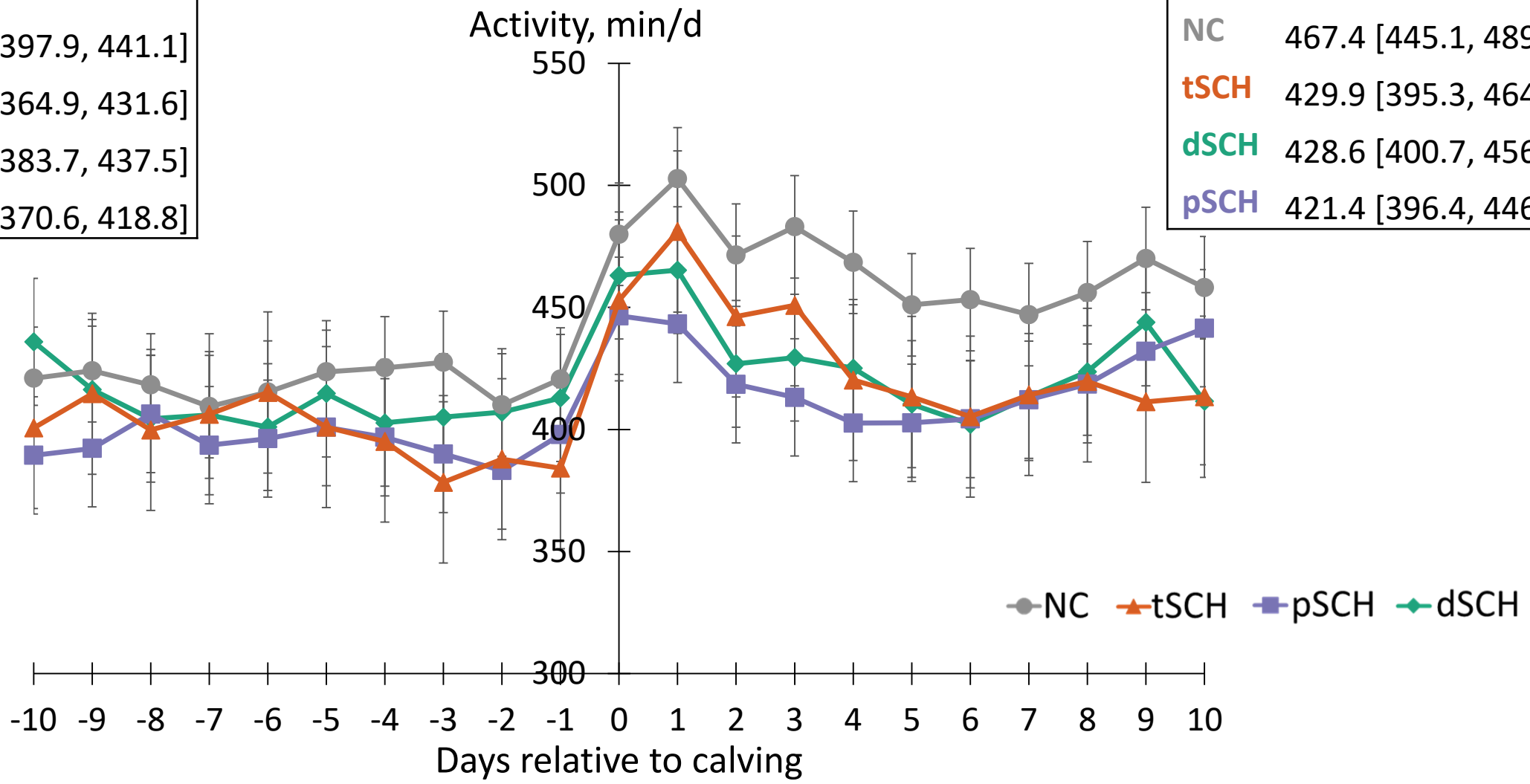
Error bars = 95% CI

Courtesy: C. Seely

Activity time & calcium dynamics

Prepartum AT, min/d	
NC	419.5 [397.9, 441.1]
tSCH	398.3 [364.9, 431.6]
dSCH	410.6 [383.7, 437.5]
pSCH	394.7 [370.6, 418.8]

Postpartum AT, min/d	
NC	467.4 [445.1, 489.7]
tSCH	429.9 [395.3, 464.5]
dSCH	428.6 [400.7, 456.5]
pSCH	421.4 [396.4, 446.4]



*error bars represent 95% CI

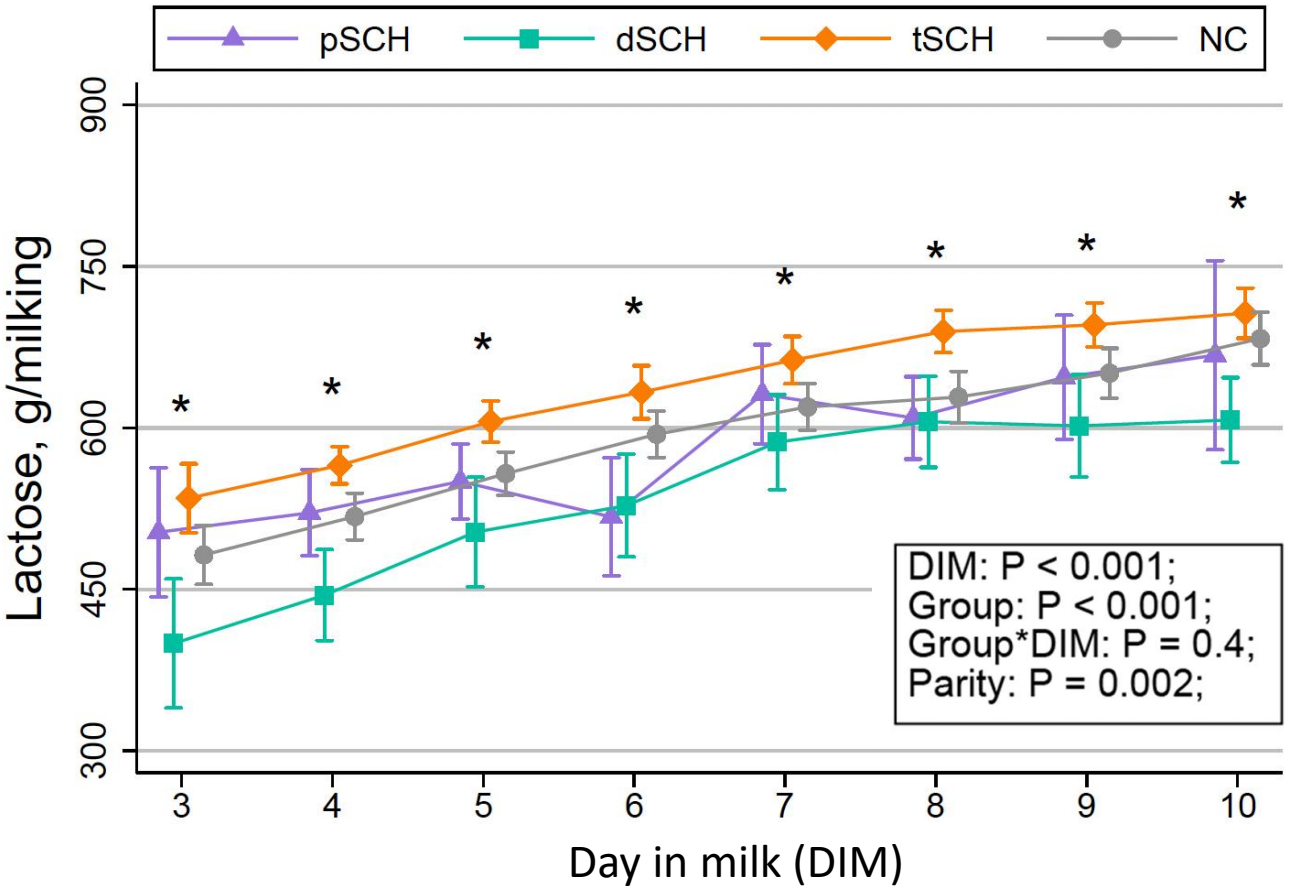
Seely & McArt; unpublished data

Milk analysis to detect hypocalcemia

- Proportional milk samples
- Fourier-transform mid infrared spectroscopy
- Measured and estimated milk constituents

Image: K. D. Bach

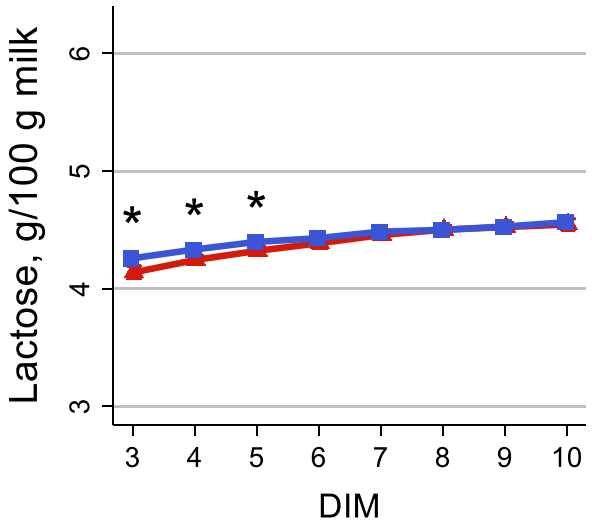
Milk analysis to detect hypocalcemia

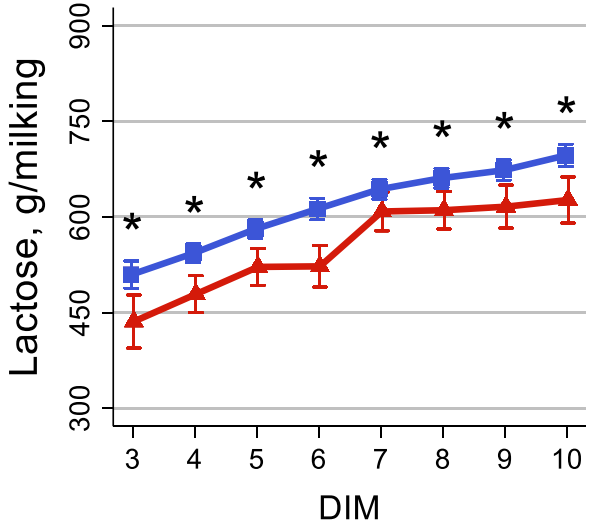


Error bars = 95% CI

Seminara et al., *JDS*, 2023

Differences in components between calcium groups





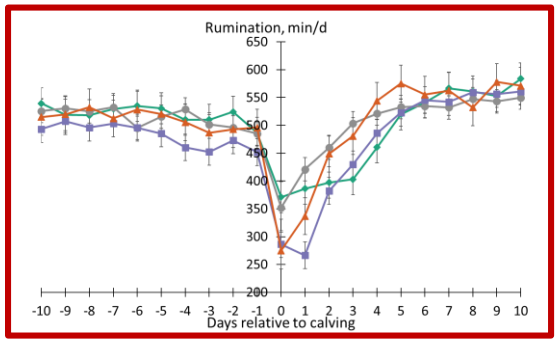
Dyscalcemic Eucalcemic

Error bars represent 95% confidence intervals.
* Differences between groups at $P < 0.05$

Seminara & McArt, unpublished data

Limitations & potential of indirect tCa measurement

Few farms and relatively small sample sizes



Deviations from normal rumination and activity time may be indicators of postpartum calcium dynamics

Proportional milk analysis might be a tool to identify cows with differing postpartum calcium dynamics

Need more cows and farms to better understand these potential applications on identifying which cows have dyscalcemia

Can we use these outcomes to assess herd-level early lactation health?

Summary

- To diagnose dyscalcemia, test for tCa at 4 d in milk in multiparous cows
- Herd-level monitoring can tell you a lot about transition management
- Expect future research on indirect calcium measurements using sensors

Acknowledgements

jmcart@cornell.edu
blogs.cornell.edu/jessmcartlab
jmcartdvm