

# Effect of induced subclinical hypocalcemia (SCH) on dry matter intake, energy status, and immune-cell function in dairy cows

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## Introduction

Objectives were to create a model to induce subclinical hypocalcemia (SCH) (blood ionized calcium [Ca<sup>2+</sup>] < 1.0 mM), and to study the effects of induced SCH on clinical parameters and function of innate and adaptive immune cells in dairy cows.

## Materials and Methods

Ten non-pregnant and non-lactating Holstein cows were blocked by lactation and assigned randomly to a normocalcemic (NC; 0.9% NaCl solution IV, plus 43 g of Ca [as Ca sulfate and Ca chloride], orally, at -1 and 11 hours) or an induced SCH (SCHI; 5% EGTA [a selective Ca<sup>2+</sup>chelator] solution at a pH of 7.4, IV) treatment group in a crossover study design. Study hour 0 referred to the initiation of IV infusion, and the infusion lasted 24 hours. The rationale for Ca supplementation in the NC treatment was that the sulfate and chloride anions present in the Ca supplement would decrease blood and urinary pH in a manner similar to that of the 5% EGTA solution. The sequence of treatments for the crossover design was either NC-SCHI or SCHI-NC, with a 6-day washout period between treatment applications, which was used to minimize carryover effects. Ionized Ca, K, Mg, and blood pH were evaluated at 0 hour and hourly during the infusion period, and at 24, 48 and 72 hours after initiation of IV infusion. Heart and respiratory rates, rectal temperature, rumen contractions, and concentrations of NEFA, BHBA, and insulin in plasma were measured at 0, 6, 12, 24, 30, 36, 48 and 72 hours after initiation of IV infusion. Dry matter intake (DMI), white blood cell differential count (WBC), neutrophil function, and lymphocyte T cell function were evaluated at 0, 24, 48 and 72 hours. Data were analyzed using PROC GLIMMIX of SAS.

## Results

Subclinical hypocalcemia was successfully induced when cows were administered the IV infusion of a 5% EGTA solution. Mean  $\pm$  SD plasma Ca<sup>2+</sup> concentration for the NC treatment was 1.27 $\pm$ 0.01 mM, whereas that for the SCHI was 0.78 $\pm$ 0.01 mM, and following the

SCHI treatment plasma Ca<sup>2+</sup> concentration returned to the reference range within 6 hours after the end of the EGTA infusion. Mean  $\pm$  SD blood pH did not differ significantly ( $P = 0.52$ ) between the SCHI (7.39 $\pm$ 0.01) and NC (7.38 $\pm$ 0.01) treatments; however, the blood pH was decreased following both treatments as a consequence of the acidifying effect of EGTA in the SCHI treatment and the supplementation with Ca chloride and Ca sulfate boluses in NC treatment. Heart and respiratory rates, rectal temperature, or WBC differential did not differ significantly between the SCHI and NC treatments. On the day of EGTA infusion, SCHI treatment cows had a significantly lower plasma K concentration (2.92 $\pm$ 0.05 mM vs 3.47 $\pm$ 0.05 mM;  $P < 0.01$ ) and higher plasma Mg concentration (0.93 $\pm$ 0.03 mM vs 0.67 $\pm$ 0.03 mM;  $P < 0.01$ ), compared with those when the cows were infused the NC treatment. Also, when administered the SCHI treatment, cows had significantly reduced DMI on the day of infusion (11.2 $\pm$ 2.2 lb [5.1 $\pm$ 0.99 kg/d] vs 22.0 $\pm$ 1.0 lb [10.0 $\pm$ 0.99 kg]/d;  $P < 0.01$ ) and decreased rumen contractions (1.7 $\pm$ 0.2 contractions/2 min vs. 2.7 $\pm$ 0.2 contractions/2 min;  $P = 0.01$ ) for the last 12 hours of the EGTA infusion. Additionally, when administered the SCHI treatment, cows had a significantly increased blood glucose (4.40 $\pm$ 0.09 mM vs 4.18 $\pm$ 0.09 mM;  $P < 0.01$ ) during the infusion period and a decreased (treatment by hour interaction) plasma insulin concentration (1.48 $\pm$ 0.25 vs 2.28 $\pm$ 0.25 ng/mL;  $P < 0.01$ ) between 6 and 18 hours after initiation of the infusion. Plasma NEFA concentrations were significantly ( $P < 0.05$ ) greater when cows were administered the SCHI treatment (0.116 $\pm$ 0.02 mM) compared with that when cows were administered the NC treatment (0.066 $\pm$ 0.01 mM). When administered the SCHI treatment, cows had a significantly ( $P < 0.01$ ) reduced percentage of neutrophils with phagocytosis (79.9 $\pm$ 8.8% of baseline vs 119.2 $\pm$ 13.0% of baseline) and oxidative burst (80.2 $\pm$ 17.9% of baseline vs 140.3 $\pm$ 17.9% of baseline) 24 hours after the end of the infusion. Furthermore, when administered the SCHI treatment, cows had significantly ( $P = 0.05$ ) reduced CD4 T cell proliferation after activation with anti-CD3 (4.9 $\pm$ 0.99 fold-change in proliferation) compared with that when cows were administered the NC treatment (9.4 $\pm$ 1.54 fold-change in proliferation).

### Significance

Induction of SCH reduced rumen contractions, decreased DMI, increased blood glucose concentration (likely because of a reduction of insulin release),

increased NEFA mobilization, and reduced neutrophil function and CD4 T cell proliferation. These findings document the negative effects of SCH on DMI, energy status, and immune-cell function in dairy cows.

## Upcoming Meetings

2014	Albuquerque	September 18 - 20
2015	New Orleans	September 17 - 19
2016	Charlotte	September 15 - 17
2017	Omaha	September 14 - 16
2018	Phoenix	September 13 - 15