Effects of an Oral Supplement Containing Calcium and Live Yeast on Circulating Calcium and Production Parameters Following I.V. Lipopolysaccharide Infusion in Dairy Cows

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Mohmmad Al-Qaisi, Sara Kvidera, Erin Horst, Carrie Shouse, Johana Mayorga, Animal Science Graduate students; Nathan Upah, Denny MacKilligan, TechMix, LLC; Dr. Leo Timms and Dr. Lance Baumgard, Professors in Animal Science

Summary and Implications

Administrating lipopolysaccharide (LPS) decreases circulating calcium (Ca) and markedly reduces both feed intake and milk yield in lactating cows. Calcium is involved in immune system activation and live yeast can increase feed intake. Whether supplemental live yeast and Ca benefits immune-challenged cows remains unclear. Therefore, study objectives were to evaluate if providing an oral supplement containing soluble Ca, live yeast and other micronutrients would ameliorate LPS-induced hypocalcemia and production parameters in lactating dairy cows.

Providing an oral supplement containing Ca and live yeast prior to and following LPS administration markedly ameliorated the LPS-induced hypocalcemia and improved DMI and milk yield. Overall, utilizing an oral supplement may be a valuable management strategy to improve animal welfare and productivity during and following immunoactivation. Additionally, infusing i.v. LPS appears to be an effective technique to model hypocalcemia and to evaluate dietary strategies aimed at increasing circulating calcium in lactating dairy cows.

Introduction

Hypocalcemia is a common metabolic disorder affecting transition dairy cows. High demand for calcium during the onset of lactation causes reduced circulating Ca. Severe hypocalcemia causes “milk fever” and requires immediate attention. Subclinical hypocalcemia is not an overt health disorder, but it has been associated with decreased productivity and other economically important phenotypes later in lactation.

Previous research showed that inflammation plays a key role in hypocalcemia, as Ca is involved in immune system activation. Immuno-activation / inflammation can be experimentally induced by infusing LPS, a cell wall component of gram-negative bacteria which elicits a well-characterized and robust immune response. Our group has previously shown that administrating LPS decreases circulating Ca and markedly reduces feed intake and milk yield.

Live yeast supplementation is thought to positively affect rumen pH, DMI, milk yield, and fermentation patterns. We hypothesized that supplementing both Ca and live yeast would benefit immune-challenged dairy cows. Therefore, study objectives were to evaluate if providing an oral supplement containing soluble Ca, live yeast and other micronutrients would ameliorate LPS-induced hypocalcemia and production parameters in lactating dairy cows.

Materials and Methods

Animals and Experimental Design: All procedures were approved by the Iowa State University Institutional Animal Care and Use Committee. Twelve non-pregnant lactating Holstein cows (269 ± 20 DIM; 760 ± 13 kg BW; 2.7 ± 0.3 parity) were utilized and housed in individual box-stalls (4.57 by 4.57 m) at the Iowa State University Dairy Farm. Cows were allowed to acclimate for 4 d during which jugular catheters were implanted. The trial consisted of 2 experimental periods (P). During P1 (3 d), cows were fed ad libitum and baseline data was collected. At the beginning of P2 (96 h) all cows were challenged i.v. with 0.375 µg/kg BW LPS (E. coli O55:B5; Sigma Aldrich, St. Louis, MO). Cows were assigned randomly to 1 of 2 treatments: 1) control (CON; no boluses; n = 6) or 2) a bolus containing Ca and live yeast, administered 0.5 pre- and 6.5 h post-LPS infusion (CLY; YMCP Vitall 44.718 g of elemental Ca; Techmix, LLC., Stewart, MN; n = 6). To isolate the effects of the oral supplement, cows were fasted for the first 12 h of P2. A stock solution of lipopolysaccride (Escherichia coli O55:B5; Sigma Aldrich, St. Louis, MO) was made at a concentration of 100 µg/mL, passed through a 0.2 µm sterile syringe filter (Thermo Scientific; Waltham, MA), and stored in a sterile glass bottle 24 h prior to P2. The total volume of LPS solution administrated was approximately 3 mL. Cows were individually fed a TMR once daily (0800 h) and orts were measured before the a.m. feeding. The TMR was formulated to meet or exceed the predicted requirements (NRC, 2001) of energy, protein, minerals, and vitamins. Cows were milked twice daily (0600 and 1800 h) during P1 and P2. Milk yield was recorded and a sample for composition analysis was obtained at each milking. Samples were stored at 4˚C with a preservative (bronopol tablet; DandF Control System, San Ramon, CA) until analysis by Dairy Lab Services (Dubuque, IA) using AOAC approved infrared analysis equipment and procedures.

Rectal temperature (Tr), and respiration rate (RR) were obtained -1, -0.5, 0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 6.5, 7, 8, 9, 10, 11, 12, 24, 48, 72, and 96 h relative to LPS infusion. Rectal temperatures were measured using a standard digital thermometer (GLA M700 Digital Thermometer, San Luis Obispo, CA). Respiration rates were determined by counting flank movements during 15 sec intervals and multiplying by four to obtain breaths per minute (bpm).

Blood samples were collected -1, -0.5, 0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 6.5, 7, 8, 9, 10, 11, 12, 24, 48, 72, and 96 h relative to LPS infusion. Circulating glucose and ionized Ca (iCa) concentrations were measured using an iStat handheld machine and cartridge (CG8+; Abbott Point of Care, Princeton, NJ).

Calculations and Statistical analysis: Area under the curve (AUC) for iCa was calculated through 96 h post-LPS by linear trapezoidal summation between successive pairs of iCa levels and time coordinates after subtracting baseline values.

Each animal's respective response variable was analyzed using repeated measures with an autoregressive covariance structure for DMI, milk yield, and milk composition and spatial power law structure for vitals and iSTAT parameters (glucose and iCa levels). The repeated effect was either day/time relative to LPS infusion. Each specific variable's pre-infusion value served as a covariate. Effects of treatments, time/day, treatment by time/day interaction were analyzed using PROC MIXED (SAS Inst. Inc., Cary, NC). Results are reported as LSmeans and were considered different when $P \leq 0.05$ and tend to differ if $P < 0.10$.

Results and Discussion

Body temperature indices: During P2, LPS administration caused mild hyperthermia (+0.21°C) relative to baseline ($P < 0.01$; Figure 1A). Likewise, respiration rate was increased 31 bpm between 0.5 to 1 h post LPS infusion ($P < 0.01$; Figure 1B) compared to baseline values; however, no treatment differences were observed in Tr and RR ($P > 0.05$; Figure 1).

Circulating iCa and glucose concentrations: Following LPS administration, circulating iCa decreased in both treatments but supplemental CLY ameliorated the hypocalcemia (469% by 48 h AUC: -10.8 vs. -1.9 mmol/L·h; $P < 0.01$; Figure 2A). LPS administration had increased (55%) circulating glucose from 0 to 2 h post LPS infusion ($P < 0.01$; Figure 2B) compared with baseline. Circulating glucose in both CON and CLY cows decreased similarly between 3 and 12 h post LPS-infusion (17%; $P = 0.40$; Figure 2B) compared to baseline.

Dry matter intake: Overall**,** LPS markedly decreased DMI (60%; $P < 0.01$) similarly for both treatments on d 1, but overall (d1-4) DMI tended to be reduced less (14 vs 30%; $P = 0.06$) in CLY supplemented vs CON cows (Figure 3A).

Milk yield and milk composition: LPS reduced ($P < 0.01$) milk yield on d 1 and 2 (48 and 61%, respectively). Overall (d 1-4), CLY supplemented cows tended ($P = 0.11$) to produce more milk (32%) following the LPS challenge and this effect was most pronounced on d 4 (20.7 vs 28.0 kg/d; $P < 0.04$; Figure 3B). Milk urea nitrogen (MUN) was decreased (11%) in CLY cows compared to CON cows ($P = 0.01$; Figure 4).

Overall Summary and Conclusion

In conclusion, providing an oral supplement containing Ca and live yeast prior to and following LPS administration markedly ameliorated the LPS-induced hypocalcemia and improved DMI and milk yield. Overall, utilizing an oral supplement may be a valuable management strategy to improve animal welfare and productivity during transition period and immunoactivation / inflammation (i.e. hospital pen implications). Furthermore, infusing i.v. LPS appears to be an effective technique to model hypocalcemia and to evaluate dietary strategies aimed at increasing circulating calcium in transitioning lactating dairy cows.

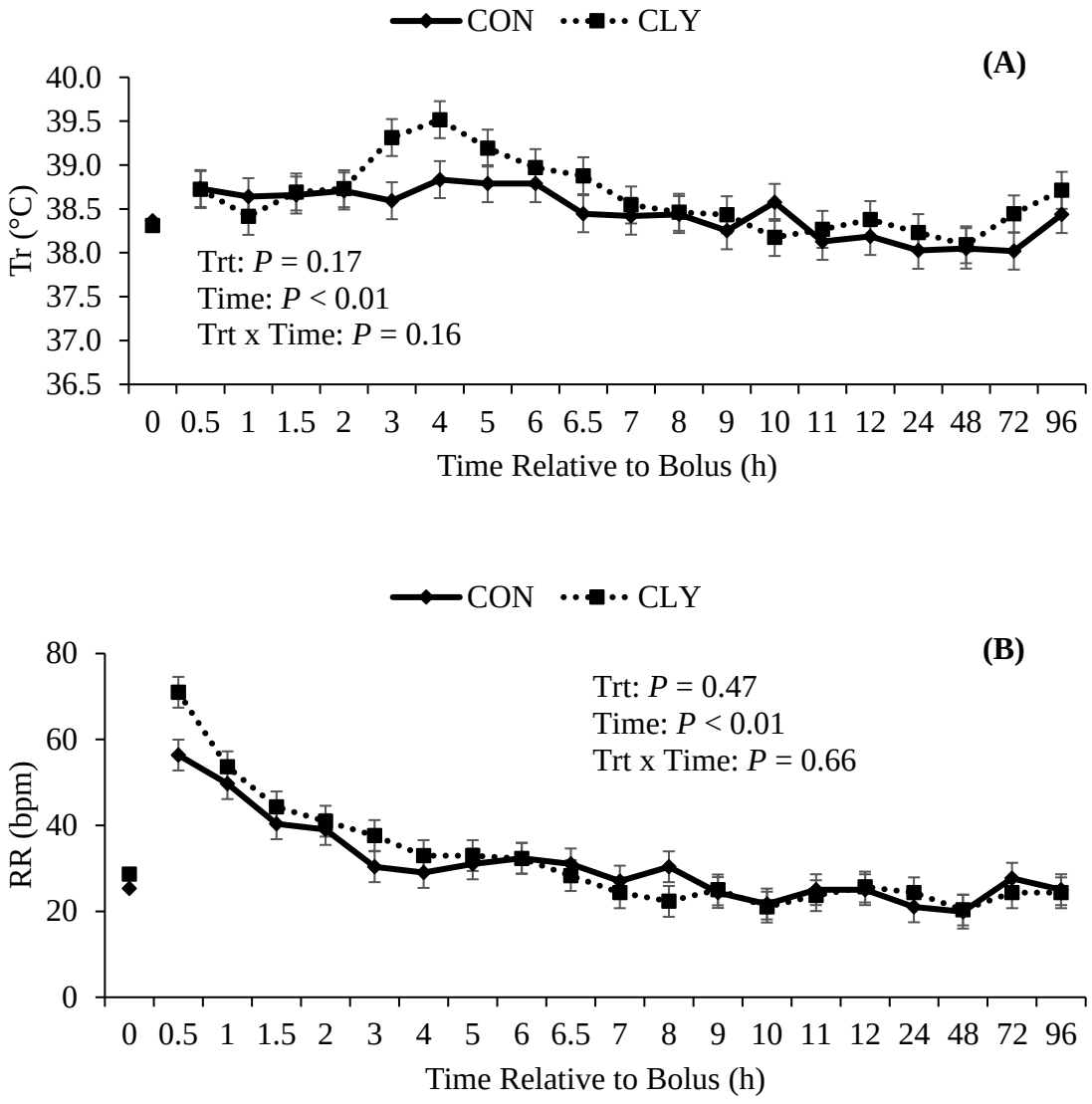

Figure 1. Effects of an oral supplement on body temperature indices in lactating dairy cows. A) Temperature (Tr), and B) Respiration rate (RR.). CON = control; CLY = calcium + live yeast bolus.

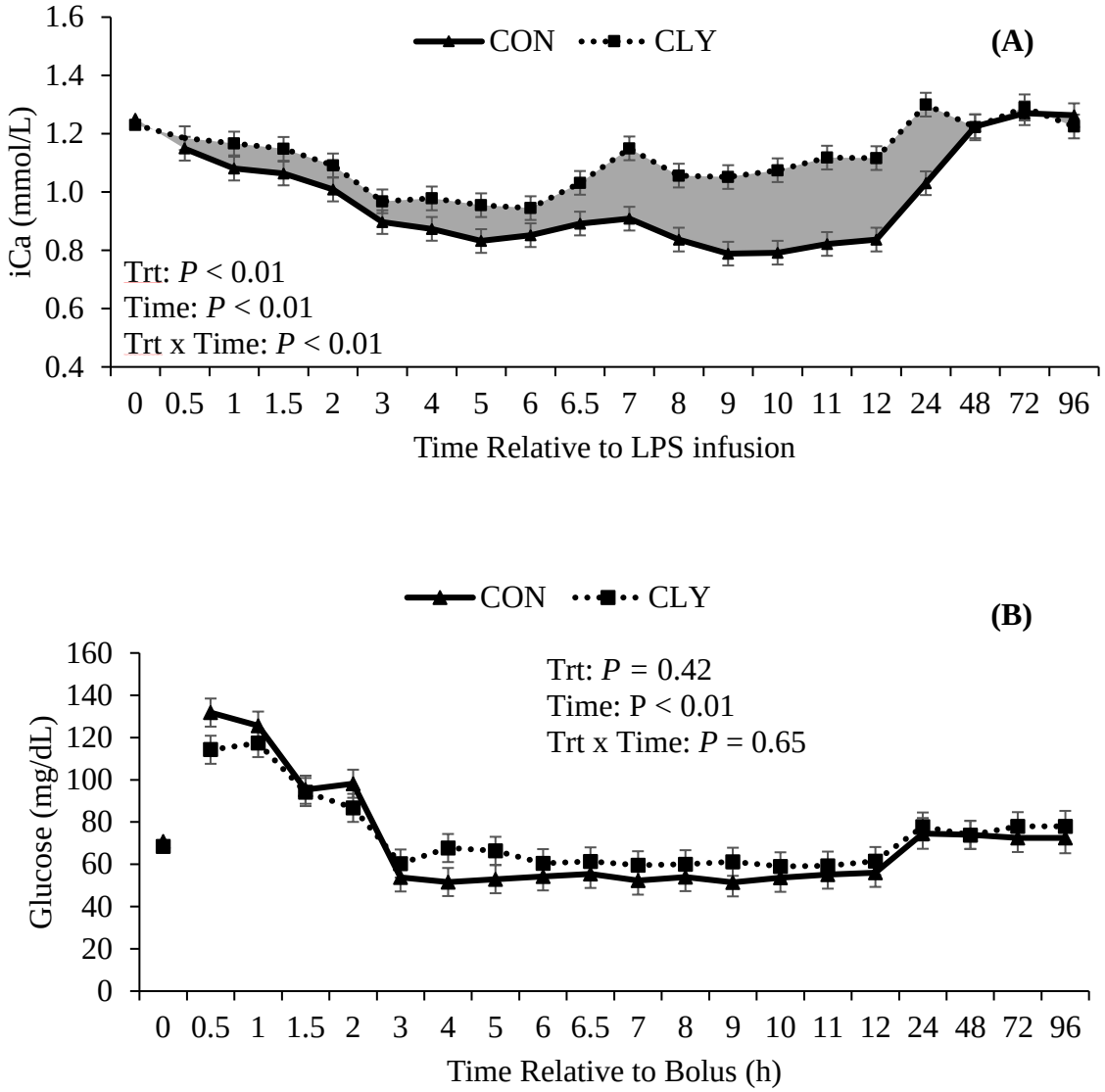

Figure 2. Effects of an oral supplement on A) circulating Ca and B) glucose in lactating dairy cows. CON = control; CLY = calcium + live yeast bolus.

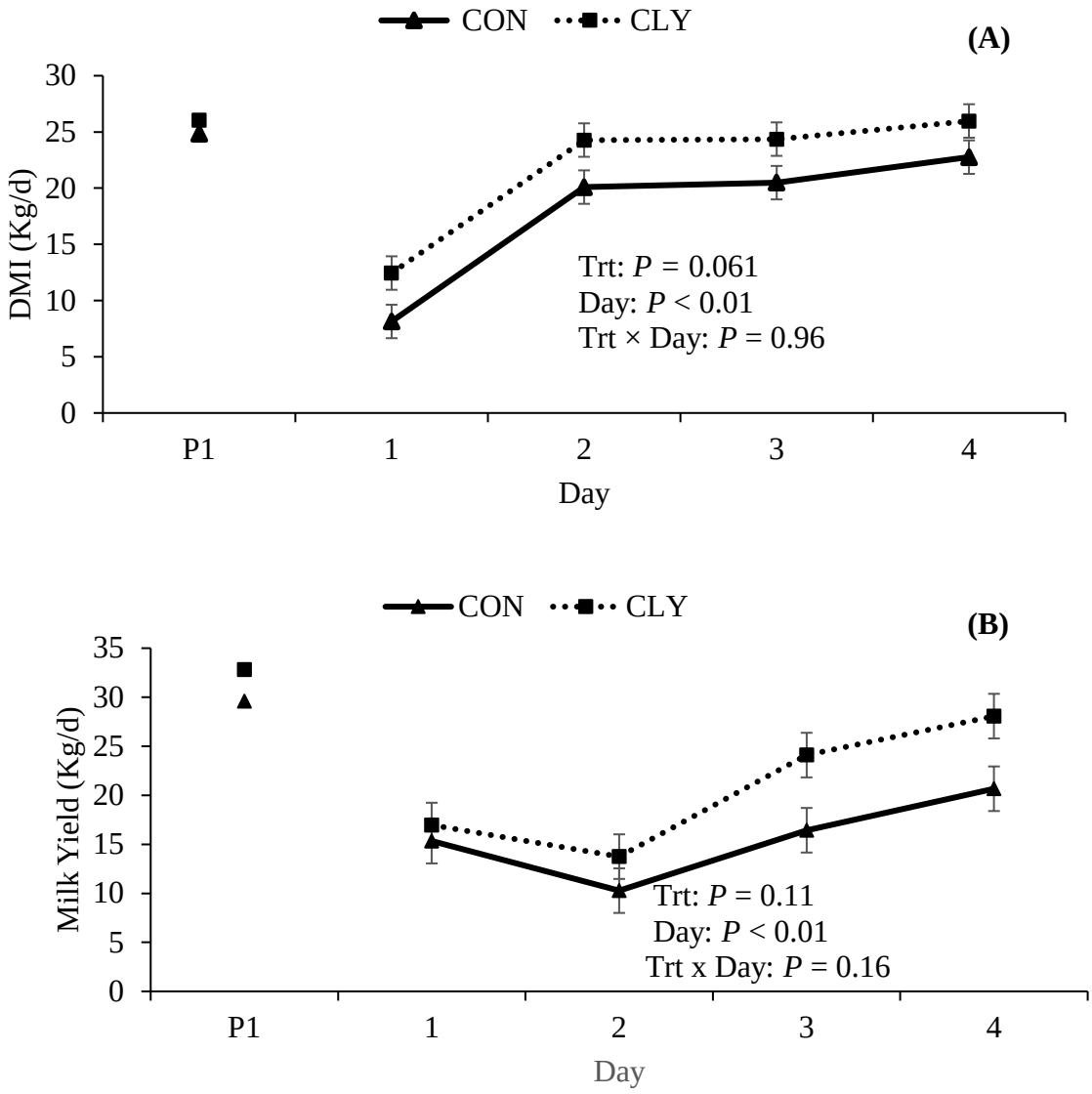

Figure 3. Effects of an oral supplement on A) DMI and B) Milk yield. The mean value from d 1 to 3 of P1 is represented by P1 on the x-axis. CON = control; CLY = calcium + live yeast bolus.

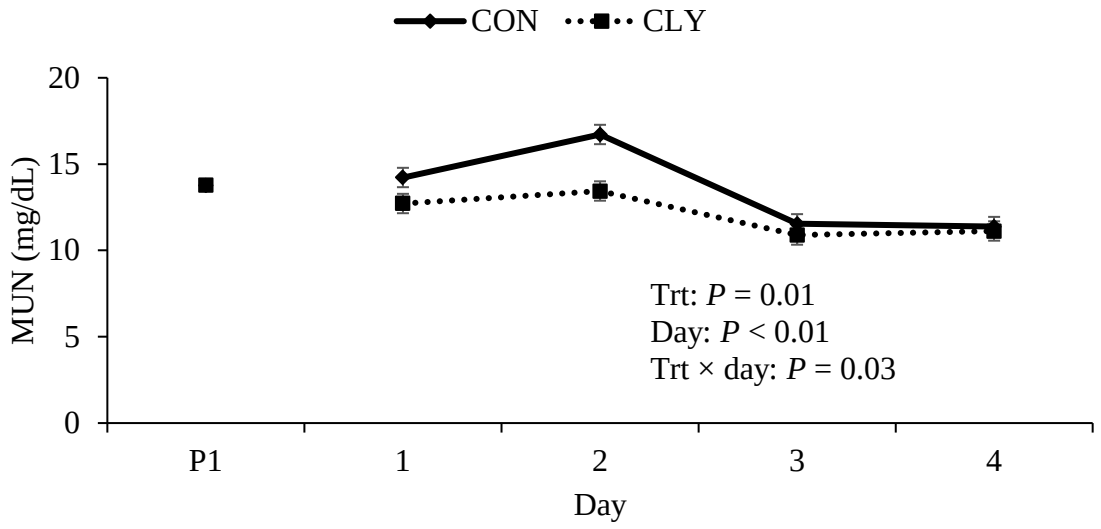

Figure 4: Effects of an oral supplement on MUN in lactating dairy cows.
CON = control; CLY = calcium + live yeast bolus.