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Effects of an oral supplement containing calcium and live yeast on post-absorptive metabolism, inflammation and production following intravenous lipopolysaccharide infusion in dairy cows

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ABSTRACT

Objectives were to evaluate the effects of an oral supplement containing soluble Ca, and live yeast in LPS-challenged dairy cows. The trial consisted of 2 experimental periods (P). During P1 (3 d), cows (n = 12) were fed ad libitum and baseline data was collected. At the beginning of P2 (which lasted 96 h), all cows were i.v. challenged with 0.375 μg/kg BW LPS. Cows were assigned randomly to 1 of 2 treatments: 1) control (CON; no bolus; n = 6) or 2) an oral bolus containing Ca and live yeast (CLY; YMCP Vitall® 44.718 g of elemental Ca; TechMix, LLC., Stewart, MN; n = 6), administered −0.5 and 6.5 h relative to LPS infusion. Following LPS administration, circulating Ca decreased in both treatments but supplemental CLY ameliorated the hypocalcemia (48 h area under the curve: −10.8 vs. −1.9 mmol/L × h; P < .01). Lipopolysaccharide decreased dry matter intake (DMI; 60%) similarly for both treatments on d 1, but overall (d 1–4) DMI tended to be reduced less (14 vs. 30%; P = .06) in CLY supplemented vs CON cows. Lipopolysaccharide reduced milk yield (70%; P < .01) from 12 to 24 h, but throughout P2, milk yield from CLY supplemented cows was increased (38%; P = .03) relative to CON cows. Overall during P2, circulating LPS-binding protein and serum amyloid A increased post LPS (3- and 4-fold, respectively, P < .01), but were unaffected by treatment (P ≥ .68). In conclusion, providing an oral supplement containing Ca and live yeast prior to and following LPS administration markedly ameliorated LPS-induced hypocalcemia and improved DMI and milk yield.

1. Introduction

Periparturient dairy cows can experience a myriad of metabolic maladies, and transient hypocalcemia represents one of the most common. Subclinical hypocalcemia is purportedly a gateway to other disorders such as ketosis, mastitis, and metritis, all of which compromise profitability and increase culling risks (DeGaris and Lean, 2008; Goff, 2008). After parturition, the mammary gland has a large calcium (**Ca**) demand, and proper parathyroid hormone (**PTH**) action is required to maintain eucalcemia (Horst et al., 2005). However, the mammary gland's Ca uptake is so acute and extensive that it often exceeds the homeostatic strategies employed to replenish circulating Ca (Goff, 2008) and cows can either enter into clinical or subclinical hypocalcemia. Although not overtly pathological, subclinical hypocalcemia has been associated with decreased productivity and other economically important phenotypes later in lactation (Goff, 2008, 2014; Oetzel, 2013). Different prophylactic and therapeutic strategies aimed at preventing post-calving hypocalcemia include: feeding pre-calving acidifying rations (-DCAD), low Ca-diets (Thilseng-Hansen et al., 2002), or Ca chelating compounds (Goff, 2008). These dietary approaches have markedly reduced clinical rates of “milk fever”, but periparturient subclinical hypocalcemia remains a frequent post-calving “pathology”. Consequently, orally bolusing Ca following parturition is a management tactic to, at least temporally (approximately 1–6 h), replenish Ca (Oetzel, 2013; Martinez et al., 2016a) and is preferable over intravenous Ca administration (Wilms et al., 2019).

Although the magnitude and extent differ, it is likely that all periparturient dairy cows (even seemingly healthy ones) experience some

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degree of inflammation (Trevisi et al., 2012; Bradford et al., 2015). Immunoactivation/inflammation can be experimentally modeled by administering lipopolysaccharide (**LPS**) and this markedly reduces blood Ca in several species (Elsasser et al., 1996; Carlstedt et al., 2000; Toribio et al., 2005; Shinozuka et al., 2018; Meurer and Hocherl, 2019) including dairy cows (Waldron et al., 2003a; Kvidera et al., 2017; Horst et al., 2018). Hypocalcemia is reported to compromise neutrophil function and thus increase susceptibility to infection (Kimura et al., 2006; Martinez et al., 2012, 2014); therefore, periparturient hypocalcemia is presumed causal to other transition cow diseases (Kimura et al., 2006; Martinez et al., 2012, 2014).

Although variable (Arambel and Kent, 1990; Kung Jr et al., 1997), live yeast and hydrolyzed yeast culture supplementation are thought to positively affect rumen pH, fermentation patterns, dry matter intake (**DMI**), and lactation performance (Desnoyers et al., 2009; Ramsing et al., 2009; Broadway et al., 2015; Bach et al., 2018) and may even benefit immune function (Broadway et al., 2015; Bach et al., 2018). Incidentally, the many of the aforementioned variables (DMI and lactation performance) are negatively affected in both poorly transitioning and experimentally immunoactivated (i.e. LPS) dairy cows (Drackley, 1999; Waldron et al., 2003b; Kvidera et al., 2017). Thus, we hypothesized that supplementing both Ca and live yeast may ameliorate the negative consequences of an activated immune system in dairy cows. Study objectives were to evaluate the effects of providing an oral supplement primarily containing soluble Ca and live yeast on circulating Ca, energetic metabolism, leukocyte dynamics and production parameters. Further, we wanted to explore if i.v. LPS administration could be used to model periparturient hypocalcemia.

2. Materials and methods

2.1. 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 (760 ± 13 kg BW; 269 ± 20 DIM; parity 2.7 ± 0.2) were utilized and housed in individual box-stalls (4.57 × 4.57 m) at the Iowa State University Dairy Farm (Ames, IA). Cows were allowed 4 d to acclimate during which they were implanted with jugular catheters as previously described (Baumgard et al., 2011). The trial consisted of 2 experimental periods (**P**). During P1 (3 d), cows were fed ad libitum and baseline data was collected (for covariate analysis). At the beginning of P2, which lasted 96 h, all cows were i.v. challenged with 0.375 μg/kg BW LPS (*Escherichia coli* O55:B5; Sigma Aldrich, St. Louis, MO). The LPS dose was selected based on the magnitude of hypocalcemia observed in a previous report (Horst et al., 2018). Cows were randomly assigned to 1 of 2 treatments: 1) control (**CON**; no bolus; $n = 6$) or 2) an oral bolus containing Ca and live yeast, administrated −0.5 and 6.5 h relative to LPS infusion (**CLY**; YMCP Vitall® TechMix LLC Stewart, MN; n = 6; 37.7 g of CaCl2 and 7.1 g of CaCO3; 30 billion cfu *Saccharomyces cerevisae,* Levucell CBCM I-1077, Lallemand SAS, Blagnac, France). A stock solution of LPS (*Escherichia coli O55:B5*; Sigma Aldrich, St. Louis, MO) was created 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.

2.2. Diet and daily measurements

Cows were individually fed a corn silage based TMR once daily (0800 h) and orts were measured before feeding. The TMR was formulated to meet or exceed the predicted requirements of energy, protein, minerals, and vitamins (NRC, 2001; Table 1). To eliminate the effects of dissimilar nutrient intake and to isolate the effects of the oral supplement, cows were fasted for the first 12 h of P2. Cows were milked twice daily (0600 and 1800 h) and yield was recorded at each milking. A milk sample for composition analysis was collected at each milking and 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 (AOAC International, 1995).

Table 1
Ingredients and composition of diet[a].

Ingredients	% of DM[b]
Corn Silage	33.6
Alfalfa Hay	13.1
Ground Corn	21.5
Mineral and Crude Protein Mix	11.9
Soybean Meal	6.1
Corn Gluten Feed	5.6
Whole Cottonseed	4.4
Molasses	1.9
Soy Plus[c]	1.9
Chemical analysis	
Starch	26.0
CP	18.2
ADF	19.3
NDF	29.5
NE_L Mcal/kg DM	1.67

[a] Values represent an average of ration nutrient summary reports collected throughout the trial. Diet dry matter averaged 50.99%.

[b] Average nutrient levels: 4.83% Fat, 0.95% Ca, 0.44% P, 0.36% Mg, 0.21% S, 1.26% K, 0.53% Na, 0.62% Cl, 78.37 mg/kg of Zn, 44.26 mg/kg of Mn, 3.51 mg/kg of Fe, 13.49 mg/kg of Cu, 0.75 mg/kg of Co, 0.32 mg/kg Se, 0.75 mg/kg of I, 14370.18 IU/kg of vitamin A, 1221.66 IU/kg of vitamin D, and 38.43 IU/kg of vitamin E.

[c] Mechanically-processed soybean meal, Dairy Nutrition Plus, Ralston, IA.

Rectal temperature (**Tr**) and respiration rate (**RR**) were obtained at −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 temperature was measured using a digital thermometer (GLA M700 Digital Thermometer, San Luis Obispo, CA). Respiration rate was determined by observing flank movements. Respiration rate was measured during a 15 s interval and were later transformed to breaths/min (**bpm**), respectively.

2.3. Blood sample collection

Blood samples (~10 mL each) were obtained daily at 0600 h during P1 and at −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 during P2. Samples were collected from the catheter and put into a tube containing K2EDTA (BD, Franklin Lakes, NJ). Plasma was harvested following centrifugation at 1500 ×*g* for 15 min at 4 °C and was subsequently frozen at −20 °C until analysis. Blood ionized calcium (**iCa**) and glucose were measured using an iStat handheld machine and cartridge (CG8+; Abbott Point of Care, Princeton, NJ) and were obtained at the same time points mentioned earlier relative to LPS infusion during P2. Administering LPS occurred immediately following the morning milking and 0 h blood sample collection. Blood samples for complete blood count (**CBC**) analysis were collected at 0, 1, 2, 3, 6, 9, 12, 24, 48, 72, and 96 h relative to LPS administration and stored at 4 °C for ~12 h before submitting to the Iowa State University's Department of Veterinary Pathology.

2.4. Sample analyses

Plasma insulin, parathyroid hormone (**PTH**), LPS-binding protein (**LBP**), and serum amyloid A (**SAA**) concentrations were determined

using commercially available kits according to manufacturers' instructions (insulin, Mercodia AB, Uppsala, Sweden; PTH, Bovine intact PTH ELISA, Immutopics, San Clemente, CA; LBP, Hycult Biotechnology, Uden, the Netherlands; SAA, Tridelta Development Ltd., Kildare, Ireland). The inter- and intra-assay coefficients of variation for PTH, LBP, and SAA were 8.4 and 11.8%, 5.4 and 8.2%, and 23.4 and 6.4%, respectively. The intra-assay coefficient of variation for insulin was 6.6%.

2.5. 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 as we have previously described (Baumgard et al., 2002). A logarithmic transformation was performed for PTH data.

Each animal's respective response variable was analyzed using repeated measures with an autoregressive covariance structure for DMI, milk yield and composition, inflammatory biomarkers, and insulin, and spatial power law structure for RR, Tr, PTH, CBC and iSTAT parameters. The repeated effect was either day/h relative to LPS infusion. Each specific variable's pre-infusion value served as a covariate. Effects of treatment, time (h or d), and treatment by time (h or d) interaction were analyzed using PROC MIXED (SAS Inst. Inc., Cary, NC). In order to compare to baseline, the overall effects period (pre vs. post-LPS infusion) and treatment (and their interaction) and cow as the random effect were also analyzed using PROC MIXED. Results are reported as least squares means and were considered different when $P \leq .05$ and a tendency if $0.05 < P \leq .10$.

3. Results

Following LPS administration, circulating iCa decreased in both treatments, but CLY ameliorated the hypocalcemia (82% by 48 h AUC: −10.8 vs. −1.9 mmol/L × h; $P < .01$; Fig. 1A). During P2, blood pH decreased (0.2%; $P = .03$; Table 2) post-LPS infusion but was not affected by treatments ($P = .19$; Table 2). Base excess, bicarbonate (**HCO_3**), and total carbon dioxide (**TCO_2**) tended to decrease (70, 9, and 8%, respectively; $P \leq .10$; Table 2) in CLY-supplemented cows, while it remained constant in CON cows relative to baseline values. Regardless of treatment, administrating LPS decreased saturated oxygen (**sO_2**; 7%; $P = .05$; Table 2) but did not influence circulating sodium, potassium, partial carbon dioxide (**pCO_2**), and partial oxygen (**pO_2**; $P > .48$; Table 2). Mild hyperthermia (+0.21 °C relative to baseline) was observed similarly in both treatments throughout P2 ($P < .01$; Fig. 1B). Respiration rate increased 31 bpm between 0.5 and 1 h post-LPS infusion ($P < .01$; data not shown) compared to baseline values. However, no effects of CLY supplementation were observed on Tr and RR ($P > .17$).

Lipopolysaccharide administration markedly decreased DMI (60%; $P < .01$) similarly for both treatments on d 1; however, overall (d1–4) DMI tended to be less reduced (14 vs. 30%; $P = .06$) in CLY supplemented cows when compared to controls (Fig. 2A). As expected, LPS reduced milk yield (70% from 12 to 24 h; $P < .01$; Fig. 2B) relative to baseline. Throughout P2, milk yield from CLY supplemented cows was increased (38%; $P = .03$) relative to CON cows (Fig. 2B). Administrating LPS increased milk fat and protein content (30 and 22%; respectively; $P \leq .03$; Table 3) in CON cows, while both milk components did not change in CLY supplemented cows relative to P1. A treatment by time interaction was detected on milk lactose content, as it decreased in both treatments relative to baseline values ($P < .01$; Table 3), but the magnitude of decrease (P1 vs. P2) was less severe in the CLY supplemented compared to the CON cows. There tended to be a treatment by time interaction on milk urea nitrogen and milk somatic cell count, as both progressively increased in both treatments for 24 and 48 h, respectively ($P < .10$; Fig. 2C and D, respectively), but peaked lower and decreased faster in the CLY supplemented compared to the controls.

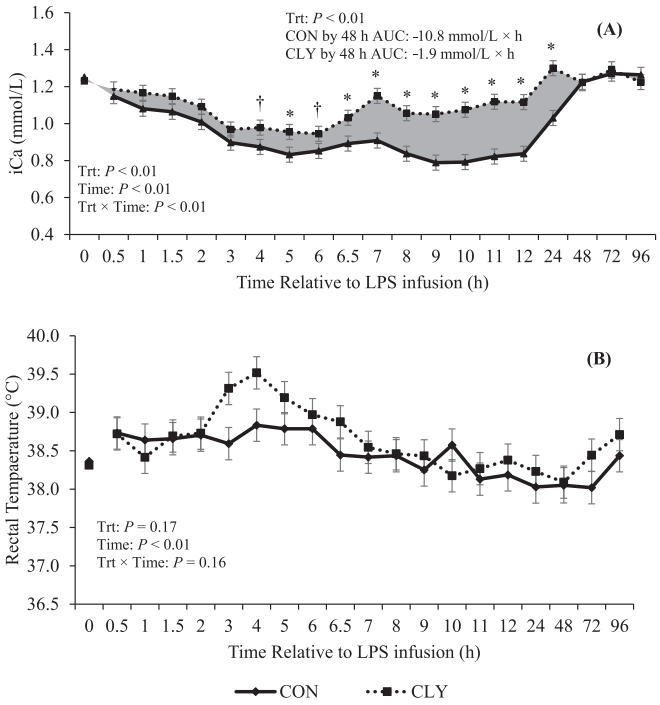

Fig. 1. Effects of an oral supplement containing calcium and live yeast on (**A**) circulating ionized calcium (iCa), and (**B**) rectal temperature in immune-challenged lactating dairy cows. Treatments: CON = control, CLY = calcium + live yeast bolus. Time 0 represents an average of measurements obtained during the baseline and used as a covariate for P2. Results are expressed as least squares means ± SEM. * denotes differences ($P < .05$) and † denotes tendencies ($0.05 < P \leq .10$).

Irrespective of treatment, infusing LPS increased (55%) circulating glucose from 0 to 2 h post-LPS administration ($P < .01$; Fig. 3A) compared with baseline values. Circulating glucose decreased in both treatments between 3 and 12 h post- LPS infusion (17%; $P < .01$; Fig. 3A) compared to baseline, but the magnitude of decrease was less severe in CLY supplemented cows relative to CON cows (10%; $P < .01$; Fig. 3A). Hyperinsulinemia was observed in both treatments at 24 h post-LPS infusion (increased 3-fold relative to baseline; $P < .01$; Fig. 3B). However, no effects of CLY supplementation were observed on circulating insulin ($P = .35$; Fig. 3B). Overall during P2, circulating PTH increased (24%; $P < .01$; P1 vs. P2; Fig. 4) post-LPS infusion relative to baseline levels; however, compared with CON, it tended to be decreased in CLY-supplemented cows during P2 (12%; $P = .10$; Fig. 4).

Overall during P2, circulating LBP and SAA increased following LPS administration (3- and 4-fold, respectively; Fig. 5A and B) relative to baseline values, but were unaffected by dietary treatment ($P \geq .68$). Administrating LPS decreased (73% for the first 3 h) circulating white blood cells (**WBC**) counts, after which cell counts progressively increased with time (35% from 12 to 48 h, relative to baseline; $P < .01$; data not shown). Circulating neutrophils exhibited a similar pattern to WBC as they initially decreased for the first 3 h following LPS administration (94%; $P < .01$; Fig. 5C). Likewise, circulating lymphocytes and monocytes initially decreased for the first 3 h following LPS administration (57 and 89%, respectively; $P < .01$) and gradually increased with time ($P < .01$; data not shown). However, no treatment differences were observed on circulating WBC, neutrophils, lymphocytes, and monocytes (Table 4).

Circulating eosinophils initially decreased (69% for 3 h; $P < .01$) post-LPS infusion and then gradually returned to baseline values with

Table 2
Effects of an oral supplement containing calcium and live yeast (CLY) on physiological parameters in immune-challenged lactating dairy cows.

Parameters	Pre-LPS infusion		Post-LPS infusion		*P*-Value			
	CON	CLY	CON	CLY	SEM	Treatment[1]	Period[2]	Treatment × Period
Base excess, mmol/L	2.67^{ab}	4.22^{a}	1.95^{ab}	1.27^{b}	0.93	0.72	< 0.01	0.06
HCO_3, mmol/L	27.44^{ab}	28.77^{a}	26.77^{ab}	26.23^{b}	0.87	0.73	< 0.01	0.08
K, mmol/L	3.9	3.9	4.0	3.9	0.1	0.85	0.78	0.48
Na, mmol/L	136.2	136.4	135.5	136.9	0.8	0.38	0.88	0.42
pCO_2, mmHg	43.26	45.00	42.93	43.73	1.47	0.49	0.48	0.68
pH	7.41	7.41	7.40	7.39	0.01	0.66	0.03	0.19
pO_2, mmHg	35.44	38.05	34.65	38.87	3.63	0.42	0.99	0.81
sO_2, %	66.94	70.00	63.11	64.96	2.66	0.45	0.05	0.79
TCO_2, mmol/L	28.72^{ab}	29.95^{a}	28.10^{ab}	27.54^{b}	0.90	0.78	< 0.01	0.10

[a-b]Values within row of each variable with differing superscripts indicate statistical difference.
[1] CON = control; CLY = calcium + live yeast bolus.
[2] Pre-LPS vs. Post-LPS infusion.

time (Fig. 6A). Relative to CON cows, CLY-supplemented cows had increased circulating eosinophils during P2 (30%; $P < .01$) and this was especially apparent after the 3rd h (Fig. 6A). Administrating LPS decreased circulating platelets in both treatments relative to baseline, and CLY supplementation alleviated this response (17%; $P = .04$) and the differences primarily existed following the 12th h (Fig. 6B). Lipopolysaccharide administration increased or tended to increase circulating hemoglobin and hematocrit (5 and 4%; $P < .05$ and $P = .10$, respectively; Table 4) relative to baseline but both metrics were unaffected by treatment. Relative to baseline, infusing LPS tended to decrease circulating red blood cells (4%; $P = .08$; Table 4), but it was not affected by treatment.

4. Discussion

It is estimated that subclinical hypocalcemia affects 25% of

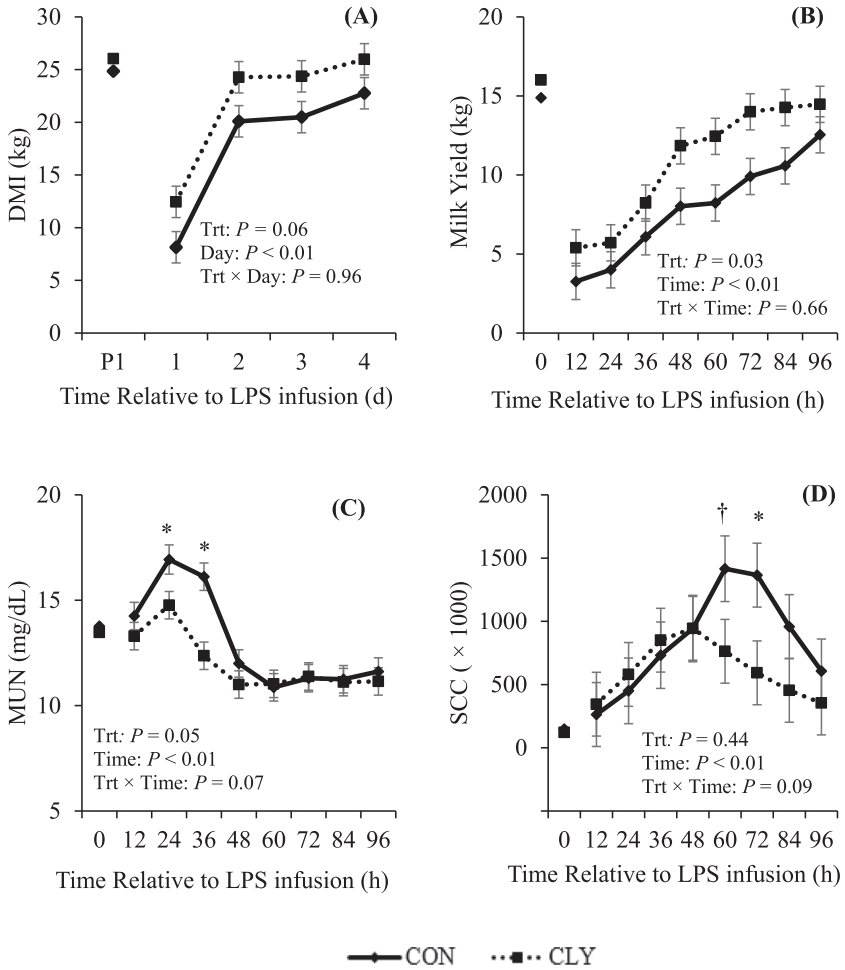

Fig. 2. Effects of an oral supplement containing calcium and live yeast on (**A**) DMI, (**B**) milk yield, (**C**) milk urea nitrogen (MUN), and (**D**) somatic cell counts in immune-challenged lactating dairy cows. Treatments: CON = control, CLY = calcium + live yeast bolus. Time 0 represents an average of measurements obtained during the baseline and used as a covariate for P2. Results are expressed as least squares means ± SEM. * denotes differences ($P < .05$) and † denotes tendencies ($0.05 < P \leq .10$).

Table 3
Effects of an oral supplement containing calcium and live yeast (CLY) on milk composition in immune-challenged lactating dairy cows.

Parameters	Pre-LPS infusion		Post-LPS infusion		*P*-Value			
	CON	CLY	CON	CLY	SEM	Treatment[1]	Period[2]	Treatment × Period
Fat, %	4.17[b]	3.64[b]	5.92[a]	4.25[b]	0.34	0.02	< 0.01	0.03
Protein, %	3.56[b]	3.13[b]	4.36[a]	3.34[b]	0.17	< 0.01	< 0.01	0.01
Lactose, %	4.62[ab]	4.58[a]	3.82[c]	4.17[bc]	0.19	0.56	< 0.01	0.01

[a-c]Values within row of each variable with differing superscripts indicate statistical difference ($P < .05$).
[1] CON = control; CLY = calcium + live yeast bolus.
[2] Pre-LPS vs. Post-LPS infusion.

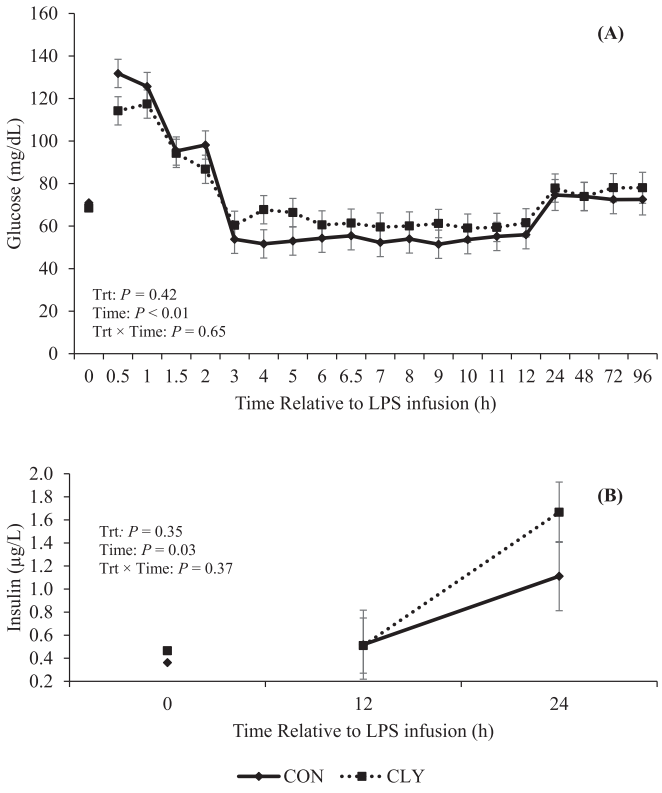

Fig. 3. Effects of an oral supplement containing calcium and live yeast on circulating (**A**) glucose, and (**B**) insulin in immune-challenged lactating dairy cows. Treatments: CON = control, CLY = calcium + live yeast bolus. Time 0 represents an average of measurements obtained during the baseline and used as a covariate for P2. Results are expressed as least squares means ± SEM.

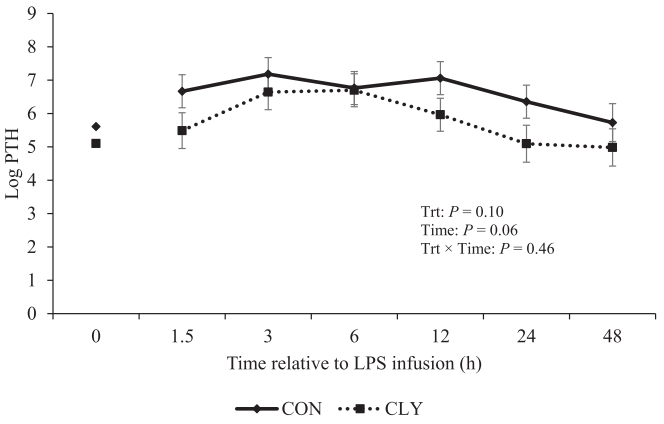

Fig. 4. Effects of an oral supplement containing calcium and live yeast on circulating parathyroid hormone (PTH) in immune-challenged lactating dairy cows. Treatments: CON = control, CLY = calcium + live yeast bolus. Time 0 represents an average of measurements obtained during the baseline and used as a covariate for P2. Results are expressed as least squares means ± SEM.

primiparous and 47–50% of multiparous periparturient dairy cows (Reinhardt et al., 2011; Oetzel, 2013). Calcium plays a key role in muscle and nerve function (Goff, 2008; Oetzel, 2013; Miltenburg et al., 2016). Thus, reduced blood Ca likely compromises skeletal muscle strength and gastrointestinal motility and is thought to be causal to poor transition cow performance (decreased DMI and milk yield, ketosis, retained placenta, etc.; Oetzel and Miller, 2012; Oetzel, 2013). In addition, previous research reported that subclinical hypocalcemia decreases insulin concentrations, which in turn allows for enhanced adipose tissue mobilization and thus increased ketone production (Martinez et al., 2012, 2014). Furthermore, all cows (even seemingly healthy ones) experience some degree of inflammation during the transition period (Trevisi et al., 2012; Bradford et al., 2015) and immunoactivation causes hypocalcemia and reduced DMI (Waldron et al., 2003a, 2003b; Kvidera et al., 2017; Horst et al., 2018) as intracellular Ca signaling plays an important role in immune cell activation (Lewis, 2001; Kimura et al., 2006). It has been shown that supplementing yeast products can improve feed intake in apparently healthy cows (Wohlt et al., 1991, 1998) and modulate immune function in transition cows (i.e., milk SCC, plasma IGG, uterine neutrophil mRNA, neutrophil populations and neutrophil function assays; Yuan et al., 2015a; Bach et al., 2018). Consequently, it is of interest to evaluate the effects of an oral supplement containing both Ca and live yeast on circulating Ca, immune system metrics, energetic metabolism and production parameters in immune-challenged lactating dairy cows.

In the current study, administrating LPS markedly increased circulating acute phase proteins (including LBP and SAA), induced mild hyperthermia, increased respiration rate, and caused leukopenia followed by leukocytosis; metrics indicating successful immunoactivation. Circulating iCa markedly decreased in both treatments following LPS infusion, but supplemental CLY partially ameliorated the hypocalcemia. The improved iCa status with the oral bolus corroborates previous experiments evaluating supplementing oral Ca in transition cows (Goff and Horst, 1993, 1994; Martinez et al., 2016a, 2016b), but disagrees with another report evaluating blood Ca following oral Ca propionate supplementation (Stokes and Goff, 2001). Reasons for the differences in circulating Ca responses following oral supplementation is not clear but may be due to differences in the amount and type of Ca salts used between experiments, or presence of a challenge (i.e. LPS), or type of Ca measured (iCa vs tCa). Although LPS-induced hypocalcemia is a well-conserved response across species (Shinozuka et al., 2018; Meurer and Hocherl, 2019) the mechanisms which mediate the response remain largely unknown, but may be attributed to leukocyte Ca uptake, which is required for immune cell activation (Lewis, 2001; Kimura et al., 2006). Leukocyte stimulation initiates a signaling cascade leading to the release of Ca from the endoplasmic reticulum into the cytosol (Lewis, 2001). Consequently, increased cytosolic Ca levels triggers Ca influx from the extracellular space via Ca^{2+} release-activated Ca^{2+} channels (Lewis, 2001), which is likely partially responsible for

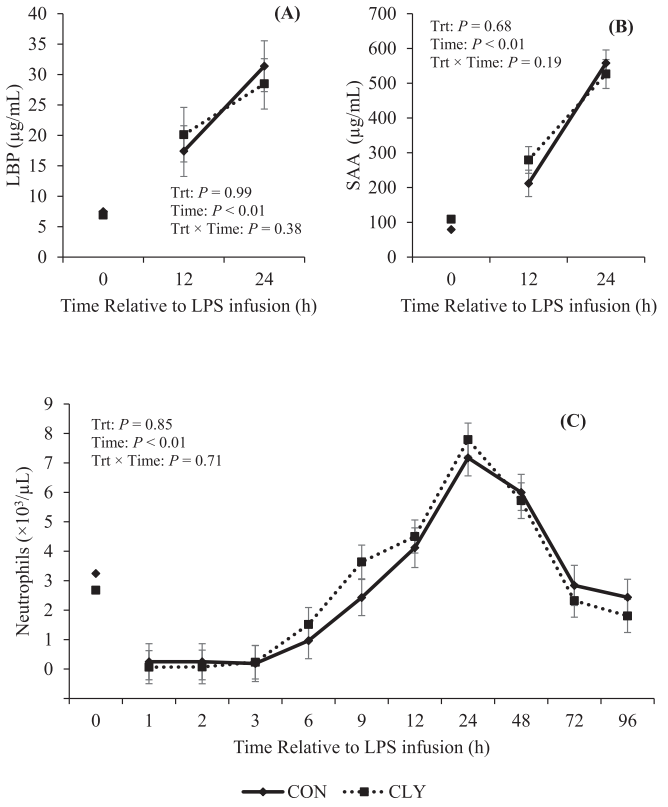

Fig. 5. Effects of an oral supplement containing calcium and live yeast on circulating (**A**) LPS binding protein (LBP), (**B**) serum amyloid A (SAA), and (**C**) neutrophils in immune-challenged lactating dairy cows. Treatments: CON = control, CLY = calcium + live yeast bolus. Time 0 represents an average of measurements obtained during the baseline and used as a covariate for P2. Results are expressed as least squares means ± SEM.

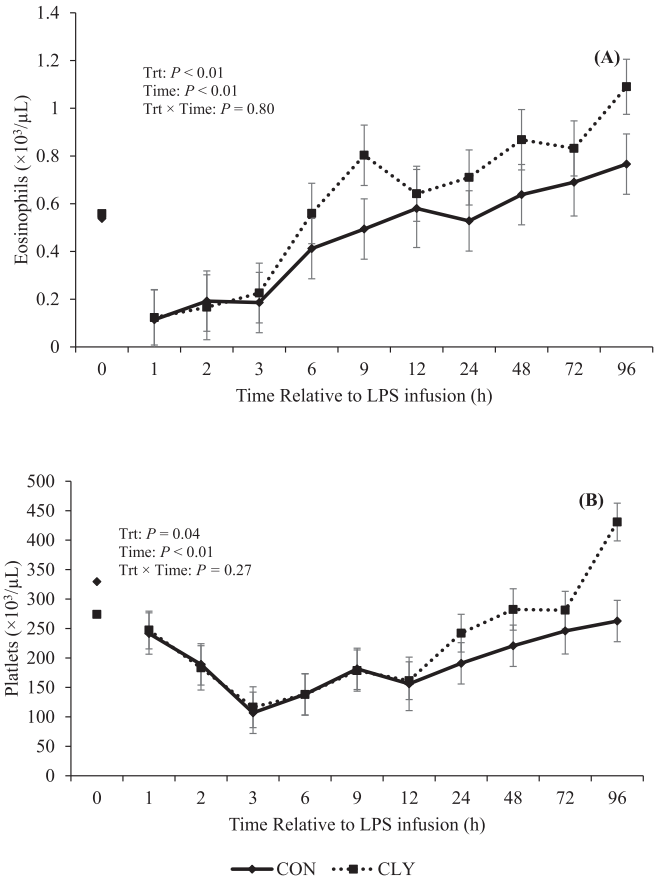

Fig. 6. Effects of an oral supplement containing calcium and live yeast on circulating (**A**) eosinophils, and (**B**) platelets in immune-challenged lactating dairy cows. Treatments: CON = control, CLY = calcium + live yeast bolus. Time 0 represents an average of measurements obtained during the baseline and used as a covariate for P2 Results are expressed as least squares means ± SEM.

systemic hypocalcemia following LPS infusion. Increased intracellular Ca plays a key role in cytokine production and cell proliferation (Kimura et al., 2006). Another possible reason for developing LPS-induced hypocalcemia could be increased Ca accumulation in both hepatic tissue and ascites fluid, which has been reported previously in pigs (Carlstedt et al., 2000). Additionally, LPS-induced hypercortisolemia may contribute to hypocalcemia as reported by Waldron et al. (2003a), as glucocorticoids act like calcitonin as highlighted by Hirsch et al. (1998). Regardless, further research is needed to identify the mechanism(s) and rationale for immune activation-triggered hypocalcemia.

Lipopolysaccharide infusion markedly decreased DMI and milk yield and this agrees with previous LPS experiments (Waldron et al., 2003b; Kvidera et al., 2017; Horst et al., 2018). Interestingly, DMI tended to be increased in CLY administered cows compared with controls. Furthermore, we observed improved milk yield in CLY compared to CON cows, which disagrees with previous Ca bolus studies in transition cows (Stokes and Goff, 2001; Oetzel and Miller, 2012; Martinez et al., 2016b). Unfortunately, specific reasons for improved production parameters (i.e. DMI, milk yield) are not clear, as we were unable to isolate the effects of Ca and live yeast. However, yeast supplementation has previously been demonstrated to positively affect rumen pH, improve nutrient utilization, DMI, fermentation patterns, and lactation performance (Desnoyers et al., 2009; Ramsing et al., 2009; Zaworski et al., 2014; Broadway et al., 2015; Bach et al., 2018). However, inconsistencies exist as others did not observe changes in DMI (Swartz

Table 4
Effects of an oral supplement containing calcium and live yeast (CLY) on complete blood cell count in immune-challenged lactating dairy cows.

Parameters	Pre-LPS infusion		Post-LPS infusion		*P*-Value			
	CON	CLY	CON	CLY	SEM	Treatment[a]	Period[b]	Treatment × Period
White blood cells, × 10^3/μL	8.1	9.1	6.3	8.9	1.8	0.42	0.48	0.56
Red blood cells, × 10^6/μL	6.2	6.3	6.62	6.4	0.3	0.87	0.08	0.33
Monocytes, × 10^3/μL	0.17	0.23	0.22	0.23	0.05	0.55	0.69	0.69
Lymphocytes, × 10^3/μL	4.1	5.7	2.9	5.0	1.4	0.34	0.19	0.80
Basophils, × 10^3/μL	0.05	0.07	0.04	0.06	0.02	0.40	0.50	0.69
Hemoglobin, g/dL	10.9	10.5	11.6	10.8	0.4	0.20	0.05	0.37
Hematocrit, %	30.3	29.6	32.1	30.0	1.0	0.28	0.10	0.30

[a] CON = control; CLY = calcium + live yeast bolus.
[b] Pre-LPS vs. Post-LPS infusion.

et al., 1994; Robinson, 1997; Yuan et al., 2015b) or milk yield following yeast product supplementation (Arambel and Kent, 1990; Yuan et al., 2015b). It is of interest to further isolate the effects of yeast and Ca on production performance in immune-challenged and transitioning dairy cows.

Overall, infusing LPS increased milk fat content, which is likely a dilution effect of severely decreased milk yield and this is consistent with our previous studies (Kvidera et al., 2017; Horst et al., 2018). Infusing LPS increased milk somatic cell count, which agrees with previous endotoxin challenge experiments (Shuster et al., 1991; Kvidera et al., 2017; Horst et al., 2018). Increased somatic cell count likely occurs because LPS impairs mammary epithelial barrier function (Wellnitz et al., 2016). Interestingly, somatic cell count tended to decrease in CLY cows relative to CON cows, indicating that either Ca or live yeast (or both) may have an immunomodulatory effect in the mammary gland. In a recent study, Yuan et al. (2015a) demonstrated that yeast supplementation decreased somatic cell linear score in transition cows. The exact mechanisms by which yeast influences immune function are not fully understood, but it could be related to the β-glucan originating from cell wall components of yeast which appears to beneficially affect the innate immune system through altered cytokine production and improved neutrophil function (as reviewed by Volman et al., 2008; Broadway et al., 2015; Bach et al., 2018).

Infusing LPS increased MUN, which is consistent with our recent i.v. LPS report (Horst et al., 2018). Reasons for increased MUN likely stems from skeletal muscle proteolysis to provide amino acids for glucose and acute phase protein production (Reeds et al., 1994) and increased hepatic AA deamination and urea synthesis is the result. Interestingly, MUN was reduced in CLY cows compared with controls, which suggests less immunoactivation-induced skeletal muscle breakdown. Another explanation may be due to the role live yeast has on improving intestinal nutrient utilization. Reducing MUN can be suggestive of increased nitrogen utilization efficiency through enhanced ammonia incorporation into microbial protein (Erasmus et al., 1992). Regardless, reduced MUN following LPS in the CLY administered cows has relevance to the transition cow and warrants further investigation.

The process of immunoactivation is energy-demanding and requires the application of homeorhetic strategies and metabolic alterations that shunt energy and amino acids towards immune cells and ultimately away from production purposes (Reeds et al., 1994; Kvidera et al., 2017). In the present study, administrating LPS caused a biphasic response in circulating glucose, with initial hyperglycemia (2 h post-LPS infusion) followed by hypoglycemia and this agrees with previous reports (Waldron et al., 2003a; Kvidera et al., 2017; Horst et al., 2018). During the hyperglycemic phase, hepatic glucose output coupled with systemic insulin resistance (at skeletal muscle and adipose tissue) likely exceeds leukocyte glucose utilization, however, with time endogenous glucose production and reduced tissue uptake is insufficient to maintain euglycemia (Lang and Dobrescu, 1991; Lang et al., 1993; Kvidera et al., 2017). Interestingly, during the hypoglycemic phase (h 3–12 post LPS), the magnitude of decrease in circulating glucose in CLY-supplemented cows was not as severe as in the controls. Reasons for this are not clear, but might suggest blunted immune activation and thus a reduced fuel need by leukocytes in the CLY-supplemented cows, as the immune system primary oxidizes glucose to meet its energetic demands (Kvidera et al., 2017). Hyperinsulinemia is another well-known response following LPS administration, and although paradoxical considering the catabolic state, it agrees with previous studies (Waldron et al., 2003b; Kvidera et al., 2017; Horst et al., 2018). This increase could be attributed to the importance of insulin for glucose uptake by immune cells (as reviewed by Baumgard et al., 2016). Supplementing CLY did not affect circulating insulin, which is surprising as Ca has a vital role in pancreatic insulin secretion (Satin, 2000; Rorsman and Ashcroft, 2018).

The parathyroid gland plays a substantial role in Ca homeostasis by secreting PTH during hypocalcemia (Patt and Luckhardt, 1942; Goff et al., 1991; Horst et al., 2005). In response to hypocalcemia, PTH increases bone resorption mechanisms, acts on the kidney to decrease Ca excretion, and stimulates the synthesis of 1,25-dihydroxyvitamin D which increases intestinal Ca absorption. All of the aforementioned actions are aimed at recovering eucalcemia (Garabedian et al., 1972; Goff, 2018). Overall, infusing LPS increased circulating PTH, which agrees with previous LPS study in horses (Toribio et al., 2005). Not surprisingly, circulating PTH from CLY supplemented cows was suppressed relative to CON, presumably due to the fact that CLY supplemented cows were not as hypocalcemic.

Administrating LPS induced leukopenia during the first 3 h post-LPS infusion and circulating neutrophils, lymphocytes, and monocytes followed a similar temporal pattern. This aligns with previous LPS studies (Griel et al., 1975; Bieniek et al., 1998; Horst et al., 2018). Leukopenia may indicate leukocyte infiltration into a site of presumed infection (Horst et al., 2018). Furthermore, administrating LPS decreased circulating platelets in both treatments relative to baseline values. In agreement with previous studies supplementing yeast (Yuan et al., 2015b), circulating platelets increased following CLY supplementation. Moreover, Kim et al. (2011) showed that supplementing yeast to neonatal vaccine-challenged calves increased platelet counts. Recent literature suggests that platelets have an essential role in modulating the inflammatory response and pathogen killing (Jenne et al., 2013). Similarly, we discovered that CLY-fed cows had increased circulating eosinophils relative to controls. This is inconsistent with Yuan et al. (2015b) who did not observe any differences in eosinophils concentrations following yeast supplementation in transition dairy cows. Reasons for increased eosinophil counts are less clear, but it could be related to the immunomodulatory effects of live yeast supplementation. Further research is needed to determine the effects of live yeast on platelets and eosinophils of immune-challenged dairy cows.

5. Conclusion

Providing an oral Ca and live yeast supplement prior to and following LPS administration markedly ameliorated LPS-induced hypocalcemia and hypoglycemia, improved DMI and milk yield, increased circulating eosinophils and platelets, and decreased circulating PTH. Collectively, it appears that CLY dampened much of the sequela associated with endotoxin exposure. Thus, utilizing an oral supplement may be a valuable management strategy to improve animal welfare and productivity during and following immunoactivation. Further research is warranted to isolate the effects of Ca (and route of administration) and live yeast on immune-challenged dairy cows.

Declaration of Competing Interest

N. Upah and D. McKilligan are TechMix employees.

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