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Short communication: The effect of calcium supplementation at calving on neutrophil function

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ABSTRACT

Low total blood calcium concentration after calving has been demonstrated to be a risk factor for reduced neutrophil function. The objective of this study was to evaluate whether administration of an injectable calcium supplement product soon after calving increased neutrophil oxidative burst or phagocytosis capacity. Cows (n = 27) from 4 farms were blocked by parity and randomly assigned to receive either calcium gluconate (35% wt/vol) in combination with calcium glucoheptonate (10% wt/vol; Theracalcium, Vétoquinol Canada Inc., Lavaltrie, Quebec, Canada) or a placebo within 12 h after calving and again 24 h later. Each dose of 120 mL was injected subcutaneously over 2 sites. Total serum calcium concentration, neutrophil oxidative burst, and neutrophil phagocytosis capacity were measured from coccygeal blood samples before (time 0) and 72 h after first treatment. There was no difference between treatment groups in lactation number, total calcium concentration, oxidative burst, or phagocytosis at time of enrollment. There was no effect of treatment on oxidative burst or phagocytosis by neutrophils. This preliminary study does not support an effect of supplemental calcium to improve neutrophil oxidative burst or phagocytosis capacity of low-parity parturient cows.
Key words: calcium supplementation, immune function, transition cow

Short Communication

In the 72 h after calving, most dairy cows have reduced blood calcium concentrations. This transitory reduction is a result of a sudden substantial demand for calcium from the plasma pool at the onset of colostrum and milk production. Initiation of homeostatic mechanisms to restore circulating blood calcium to normal levels take several days, with the nadir of blood calcium concentrations occurring 12 to 24 h following parturition (Goff, 2008).

Ionized calcium is important for messaging in cell metabolism and proliferation through changes in cytosolic ionized calcium concentrations. Intracellular calcium signaling is a key element in immune cell activation through influx of calcium from the extracellular space when antigen receptors are triggered (Vig and Kinet, 2009). In vitro, low extracellular ionized calcium was associated with decreased phagocytosis in bovine neutrophils (Ducusin et al., 2003). Kimura et al. (2006) demonstrated that mononuclear cells of periparturient cows have lower intracellular calcium stores, resulting in blunted calcium release in response to immune cell activation signals. Reduced calcium release in response to an immune cell activation signal likely contributes to periparturient immune suppression, but the reduced immune function that is experienced by almost all transition dairy cows with varying severity (Kehrli et al., 1989) is multifactorial and not well understood.

A decrease in circulating neutrophil oxidative burst activity postpartum has been found in cows that develop metritis (Hammon et al., 2006) and endometritis (Mateus et al., 2002; Hammon et al., 2006). Similar to other immune cells, an increase in intracellular calcium concentration is an early event in neutrophil activation (Burgos et al., 2011). Martinez et al. (2012) found neutrophil number, oxidative burst, and phagocytosis capacity to be reduced among cows with a blood calcium <2.15 mmol/L between 1 and 3 DIM. These cows were also at greater risk of metritis. Supporting neutrophil function to reduce postpartum metabolic and reproductive disease is desirable, but it is unclear to what extent prevention or treatment of hypocalcemia may contribute. Prophylactic calcium supplementation, which is commonly administered to parturient dairy cows to reduce the incidence of clinical hypocalcemia, may be one way to achieve this. The objective of this study was to evaluate whether administration of an injectable calcium supplement product soon after calving increased neutrophil oxidative burst or phagocytosis capacity.

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A randomized controlled trial was conducted using parturient cows from 4 commercial dairy farms in Ontario, Canada, in June 2014. Herds were purposively selected based on proximity to the University of Guelph, willingness to comply with the calcium supplementation protocols, and herd size such that there would be cows available to enroll that had calved within the 12 h before the technician's visit. Producers agreed to refrain from using other forms of prophylactic calcium supplementation in enrolled cows and consented to a study protocol that had been reviewed and approved by the University of Guelph Animal Care Committee. All herds fed a TMR and prepartum cows did not receive anionic dietary supplements. The sample size for this proof-of-concept study was 13 cows per treatment based on detecting a 10% point difference (SD = 9) in mean proportion of neutrophils performing oxidative burst.

Cows including first-parity animals that had calved in the previous 12 h were enrolled on the first day of the week for 3 consecutive weeks. Cows that showed signs of milk fever or injury related to calving or had already received a calcium supplementation product were excluded. Cows were randomly assigned to receive the calcium supplementation product or a placebo. Technicians administered and recorded experimental treatments according to randomized assignment sheets and were blinded to the treatment given. Cow ID, parity, calving ease, and time of calving were collected from farm personnel.

Prior to receiving treatment or the placebo, a single whole-blood sample was collected for calcium and neutrophil analysis. Whole blood was collected from the coccygeal vessels using a 20-gauge, 1-inch hypodermic needle into sterile glass 10-mL blood collection tubes without anticoagulant (BD Vacutainer Precision Glide; Becton Dickinson, Franklin Lakes, NJ). An additional 2 tubes were collected with the anticoagulant acid citrate dextrose (Vacutainer, Becton Dickinson).

Cows in the treated group received calcium gluconate (35% wt/vol) in combination with calcium glucoheptonate (10% wt/vol) for a total of 9.46 g of calcium (Theracalcium, Vétoquinol Canada Inc., Lavaltrie, Quebec, Canada) given in 2 doses (within 12 h after calving at enrollment and again 24 h later). Each dose was 120 mL injected over 2 sites (60 mL per site) subcutaneously. Cows in the control group received a similar volume of placebo (medication vehicle solution with no active ingredient) at time of enrollment and 24 h later. At 3 DIM, the blood sampling procedure was repeated such that measurement of blood calcium and neutrophil analyses were completed twice per animal. Within 3 h of collection, serum was separated by centrifugation at 1,500 × *g* for 15 min. Serum was stored at −20°C and analyzed at the Animal Health Laboratory, University of Guelph. Total calcium (**tCa**) concentration was measured using the Cobas Calcium Gen 2 kit (Roche Diagnostics, Indianapolis, IN). The analytical sensitivity of the calcium assay was 0.2 mmol/L, and the interassay control coefficient of variation was 1.49%.

Neutrophil isolation from whole blood with acid citrate dextrose was done within 3 h of collection. The procedure for neutrophil isolation and the oxidative burst and phagocytosis assays was as reported in Miltenburg et al. (2018). For the oxidative burst assay, 200 μL of reconstituted neutrophils was incubated with fluorescent dye, and oxidative burst was stimulated with phorbol myristate acetate (**PMA**). Each sample had a negative control of neutrophils incubated without PMA. For the phagocytosis assay, 200 μL of reconstituted neutrophils was incubated with 1×10^6 fluorescently labeled 1-μm beads (TransFluo-Spheres Fluorescent Microspheres, Molecular Probes, Thermo Fisher Scientific, Waltham, MA) and 50 μL of activated normal cow serum with Zymosan A (Sigma-Aldrich, St. Louis, MO). Each sample had a negative control of neutrophils incubated without fluorescent beads.

Neutrophil fluorescence was measured on a flow cytometer (FACScan, Becton Dickinson) with Cell Quest software (Becton Dickinson). A gate was placed around the neutrophil population on forward versus side scatter cytograms using FlowJo software (Tree Star, Ashland, OR). Per Miltenburg et al. (2018), the shift in the percentage of cells that underwent oxidative burst or phagocytosis was evaluated relative to each cow's negative control. A gate was placed around ≥97% of negative controls fluorescence (no PMA for the oxidative burst analysis and no fluorescent beads for the phagocytosis analysis), and the difference between the negative control and the positive observation for the percentage of cells outside the negative control gate was used to express success of oxidative burst or phagocytosis.

All statistical analyses were performed in SAS (version 9.4; SAS Institute, Cary, NC). Descriptive statistics were generated using the PROC MEANS and PROC FREQ procedures of SAS. The outcomes of interest were the difference between the samples and negative control for the mean percentage shift in cells that successfully performed oxidative burst and phagocytosis at 3 DIM. The oxidative burst variable required a natural logarithmic transformation to normalize the data with an adjustment factor of 0.5. Mixed linear regression models (MIXED procedure in SAS) were built for oxidative burst and phagocytosis with an autoregressive covariance structure. Farm, parity (primiparous or multiparous), and total calcium concentration at the time of enrollment as a continuous variable and a di-