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Glucose supplementation has minimal effects on blood neutrophil function and gene expression in vitro

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ABSTRACT

During early lactation, glucose availability is low and the effect of glucose supply on bovine polymorphonuclear leukocyte (PMNL) function is poorly understood. The objective of this study was to determine the effect of glucose supplementation on the function and transcriptomic inflammatory response of PMNL from cows in early and mid-lactation in vitro. Twenty Holstein cows in early (n = 10; days in milk = 17 ± 3.1) and mid-lactation (n = 10; days in milk = 168 ± 14.8) were used for this study. Jugular blood was analyzed for serum concentrations of nonesterified fatty acids, β-hydroxybutyrate, and glucose. Polymorphonuclear leukocytes were isolated and diluted using RPMI (basal glucose concentration was 7.2 m*M*) to different concentrations of PMNL/mL for phagocytosis, chemotaxis, gene expression, and medium analyses. Working solutions of glucose (0 or 4 m*M* of D-glucose) and lipopolysaccharide (0 or 50 μg/mL) were added and tubes were incubated for 120 min at 37°C. Media were analyzed for concentrations of glucose and tumor necrosis factor-α (TNF-α). Data were analyzed in a randomized block (stage of lactation) design. Challenge with lipopolysaccharide increased the expression of the genes encoding for nuclear factor kappa B (*NFKB1*), IL-10 (*IL10*), *IL1B*, *IL6*, *IL8*, TNF-α (*TNFA*), glucose transporter 3 (*SLC2A3*), and the concentration of TNF-α in medium (147.3 vs. 72.5 pg/mL for lipopolysaccharide and control, respectively). Main effect of stage of lactation was minimal where the expression of *IL10* increased for cows in early compared with cows in mid-lactation. After lipopolysaccharide challenge, cows in early lactation experienced more marked increases in the expression of *IL6*, *TNFA*, and *IL8* when compared with cows in mid-lactation. Glucose supplementation had minimal effects on gene expression where glucose supplementation increased the expression of lysozyme (*LYZ*). Glucose supplementation increased PMNL phagocytosis but did not alter chemotaxis, morphology, or concentration of TNF-α in the medium. Under the conditions of the experiment, stage of lactation had minimal effects on PMNL response to glucose supply where only the expression of *NFKB1* and the production of TNF-α were greater for cows in mid-lactation when compared with early lactation. Metabolic profiles for cows in early lactation did not parallel those for cows during the early postpartum period and may partly explain results for this study. Future studies investigating the effect of glucose supply on bovine PMNL function in vivo and how this may be altered by stage of lactation are warranted.
Key words: glucose, inflammation, lactating cow, neutrophil

INTRODUCTION

Inflammation of the mammary gland (i.e., mastitis) is usually associated with the presence of a pathogen from either gram-positive or gram-negative bacteria (Kehrli and Shuster, 1994). During mastitis, circulating PMNL are of key importance in controlling the severity and duration of mastitis, accounting for approximately 95% of somatic cell population in milk (Kehrli and Shuster, 1994; Burvenich et al., 2007). Most periparturient cows mobilize body tissue to meet the nutrient demands for lactation and hence experience some degree of negative energy balance, characterized by changes in circulating NEFA, BHBA, and glucose (Drackley et al., 2006). The metabolites associated with degree of negative energy balance, such as circulating BHBA and NEFA, can impair several aspects of the immune response (Ingvartsen and Moyes, 2013). Coupled with natural immunosuppression, as well as changes in the hormonal, neurological, and digestive systems, cows are at high risk of development of diseases, especially mastitis, during early lactation (Ingvartsen, 2006).

Studies in humans and rodent models have revealed that glucose is the preferred fuel for phagocytic cells

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such as macrophages and PMNL (Newsholme et al., 1987; Newsholme and Newsholme, 1989; Healy et al., 2002; Pithon-Curi et al., 2004). Indeed, activated phagocytic cells supplemented with glucose showed enhancement of metabolism by increasing the production of lactate, ATP, and activity of enzymes involved in glycolysis, the pentose phosphate pathway, and the citric acid cycle (Newsholme and Newsholme, 1989; Spolarics et al., 1991; Healy et al., 2002; Pithon-Curi et al., 2004). In addition, glucose supplementation enhanced phagocytosis and the production of reactive oxygen species in phagocytic cells (Barghouthi et al., 1995; Furukawa et al., 2000). During the inflammatory response, research has shown that systemic metabolic changes occur including increased circulating glucose concentrations in lactating dairy cows (Moyes et al., 2014) and nonlactating heifers (Steiger et al., 1999). Hence, glucose may be spared for utilization by phagocytic cells, such as PMNL, rather than the synthesis of milk components in the mammary gland as proposed by Gross et al. (2014). During early lactation, glucose availability is low and may partly explain the natural immunosuppression observed at this time. The hypothesis of the present study was that additional glucose will improve the metabolic and inflammatory response of bovine blood PMNL after LPS challenge and that supplemental glucose will improve PMNL response for cows in early lactation when compared with cows in mid-lactation. The objective of this study was to determine the effect of glucose supplementation on the inflammatory and metabolic response of PMNL for cows in early and mid-lactation in vitro.

MATERIALS AND METHODS

All procedures involving the use of live animals were approved in accordance with the regulations and guidelines set forth by the USDA Beltsville Animal Care and Use Committee.

Animals

Fourteen primiparous and 7 multiparous ($\geq$2 lactations) Holstein cows in early (n = 10; DIM = 17 $\pm$ 3.1; 34.2 kg of milk/d) and mid-lactation (n = 10; DIM = 168 $\pm$ 14.8; 37.8 kg of milk/d) were used for this study. All cows were free from clinical signs of disease before sample collection for this study with median composite milk SCC of 107,000 cells/mL. All cows were housed and fed in free stalls, had free access to water, and were milked twice daily at 0600 and 1800 h. At 0730, 1400, and 1730 h, cows were fed ad libitum daily a TMR formulated to provide the NRC (2001) requirements (NE_L = 1.65 Mcal/kg of DM, CP = 17.2% of DM) of lactating dairy cows averaging 100 DIM and producing 40 kg of milk/d with BW and BCS of 610 kg and 2.5, respectively.

Jugular blood (~150 mL) was collected from each cow after the morning milking and before the morning feeding. Blood was collected into vacutainer tubes containing acid-citrate dextrose (Fisherbrand, Thermo Fisher Scientific Inc., Pittsburgh, PA), inverted to mix and placed on ice. Blood samples were processed within 1 h after collection. In addition, blood (~10 mL) was collected without anticoagulant (Fisherbrand, Thermo Fisher Scientific Inc.). Blood containing no anticoagulant was allowed to clot at room temperature for ~2 h. Tubes were centrifuged at 2,000 $\times$ *g* for 15 min at 4°C (model 5810R, Eppendorf, Hauppauge, NY) and media were frozen at −20°C for metabolite analysis.

Isolation of PMNL

Isolation of polymorphonuclear leukocytes, viability, and cell differentiation were performed using endotoxin free materials and reagents, according to the procedures described by Garcia et al. (2015). Briefly, after blood centrifugation, plasma and one-third of the red blood cells (**RBC**) were discarded and the remaining RBCs were lysed with ice-cold water and isotonicity was restored using PBS. The pellet was washed 3 times in 10 mL of PBS and resuspended in 1 mL of calcium- and magnesium-free Hanks' buffered saline solution (**CMF-HBSS**; Sigma-Aldrich Co., St. Louis, MO). Concentrations of PMNL were measured using a TC-20 automated cell counter (Bio-Rad Laboratories Inc., Hercules, CA). The viability of PMNL was determined using the trypan blue (0.1%, Bio-Rad Laboratories Inc.) exclusion method, resulting in an average viability of 94%. Cell differentials were determined microscopically on cytospin preparations using a commercially available hematology staining kit (Hema-FastTM 3-Step Hematology Staining Kit; Fisherbrand, Thermo Fisher Scientific Inc.), indicating that 85% of the cells were PMNL.

PMNL Morphology

Morphology of isolated blood PMNL (n = 8/stage of lactation) was performed according the procedure described by Moyes et al. (2009). Briefly, 5 fields per cow were evaluated for PMNL morphology. The PMNL were classified as either mature or immature (banded or juvenile) as described by Paape et al. (2003). The percentage of mature PMNL was calculated from the average of the 5 fields per cow and subjected to statistical analysis.