J. Dairy Sci. 98:1–14
http://dx.doi.org/10.3168/jds.2014-8811

Prepartal dietary energy level affects peripartal bovine blood neutrophil metabolic, antioxidant, and inflammatory gene expression

Z. Zhou,* D. P. Bu,†‡§ M. Vailati Riboni,* M. J. Khan,* D. E. Graugnard,* J. Luo,# F. C. Cardoso,* and J. J. Loor*[1]
*Department of Animal Sciences and Division of Nutritional Sciences, University of Illinois, Urbana 61801
†State Key Laboratory of Animal Nutrition, CAAS-ICRAF Joint Laboratory on Agroforestry and Sustainable Animal Husbandry, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, 100193, P. R. China
‡World Agroforestry Centre, East and Central Asia, Beijing 100081, China
§Synergetic Innovation Center of Food Safety and Nutrition, Harbin, 150030, China
#College of Animal Science and Technology, Northwest A&F University, Yangling, Shaanxi, 712100, P. R. China

ABSTRACT

During the dry period, cows can easily overconsume higher-grain diets, a scenario that could impair immune function during the peripartal period. Objectives were to investigate the effects of energy overfeeding on expression profile of genes associated with inflammation, lipid metabolism, and neutrophil function, in 12 multiparous Holstein cows (n = 6/dietary group) fed control [CON, 1.34 Mcal/kg of dry matter (DM)] or higher-energy (HE, 1.62 Mcal/kg of DM) diets during the last 45 d of pregnancy. Blood was collected to evaluate 43 genes in polymorphonuclear neutrophil leukocytes (PMNL) isolated at −14, 7, and 14 d relative to parturition. We detected greater expression of inflammatory-related cytokines (*IL1B, STAT3, NFKB1*) and eicosanoid synthesis (*ALOX5AP* and *PLA2G4A*) in HE cows than in CON cows. Around parturition, all cows had a close balance in mRNA expression of the pro-inflammatory *IL1B* and the anti-inflammatory *IL10*, with greater expression of both in cows fed HE than CON. The expression of *CCL2*, *LEPR*, *TLR4*, *IL6*, and *LTC4S* was undetectable. Cows in the HE group had greater expression of genes involved in PMNL adhesion, motility, migration, and phagocytosis, which was similar to expression of genes related to the pro-inflammatory cytokine. This response suggests that HE cows experienced a chronic state of inflammation. The greater expression of *G6PD* in HE cows could have been associated with the greater plasma insulin, which would have diverted glucose to other tissues. Cows fed the HE diet also had greater expression of transcription factors involved in metabolism of long-chain fatty acids (*PPARD, RXRA*), suggesting that immune cells might be predisposed to use endogenous ligands such as nonesterified fatty acids available in the circulation when glucose is in high demand for milk synthesis. The lower overall expression of *SLC2A1* postpartum than prepartum supports this suggestion. Targeting interleukin-1β signaling might be of value in terms of controlling the inflammatory response around calving. The present study revealed that overfeeding cows during late pregnancy results in activation, ahead of parturition, of PMNL responses associated with stress and inflammation. These adaptations observed in PMNL did not seem to be detrimental for production.
Key words: dietary energy, polymorphonuclear neutrophil, gene expression

INTRODUCTION

On average, 16.5% of cows in the US dairy cattle industry experience clinical mastitis (USDA, 2008). Once a pathogen is detected by the receptors in the epithelial cells of the mammary gland, the acute-phase reaction begins and the immune system is activated to eliminate the pathogen (Oviedo-Boyso et al., 2007). Polymorphonuclear neutrophil leukocytes constitute up to 70% of the circulating white blood cells in sick bovine compared with 30 to 40% in healthy animals (Goldsby et al., 2000). In fact, after initiation of an inflammatory response during an infection, PMNL provide the first line of defense against the invading pathogens, especially those causing mastitis (Paape et al., 2003) and become the predominant cell type observed (Sordillo et al., 1997).

During the transition period, the increased nutrient demand, the decrease in DMI, and the drastic changes in endocrine status (e.g., increased estrogen) can give rise to metabolic disorders and suppressed functions of immune cells (Grummer, 1995; Mallard et al., 1998). The latter are characterized by impaired neutrophil

Received September 3, 2014.
Accepted April 8, 2015.
[1]Corresponding author: jloor@illinois.edu

trafficking, phagocytosis, and killing capacity (Kehrli et al., 1989), which could eventually lead to increased susceptibility to mammary infections. In addition, the cytokine and hormonal changes (e.g., glucocorticoids) in the transition period are also closely linked to neutrophil development and immunity-related activities, the mechanism of which remains to be elucidated (Burton et al., 2005).

The level of energy consumption prepartum also may be a determinant factor for inflammatory status of cows. For instance, feeding higher-grain diets during the dry period may lead to overconsumption of energy from carbohydrates (Dann et al., 2006). One week postpartum, serum NEFA concentrations were greater in cows fed high-energy diets ad libitum than in cows fed a control diet with more straw to help regulate gut fill and maintain consistent intake (Khan et al., 2014).

At chronically elevated concentrations, NEFA led to a reduction in PMNL viability, which could impair the immune response to pathogens (Scalia et al., 2006). Although PMNL function can be measured through traditional neutrophil function tests, gene expression technology enables us to explore how PMNL cells respond to the intensity of lipid mobilization as a way to understand effects of dry-period nutritional management on immune cell function at a molecular level.

We hypothesized that a high level of dietary energy intake induces body deposition and high concentrations of NEFA postpartum, serving in part to alter PMNL gene expression during the periparturient period. Specifically, the objectives were to determine gene expression of 43 inflammatory and lipid metabolism markers (Table 1) in PMNL in cows fed a high- or low-energy level diet in the prepartum period.

MATERIALS AND METHODS

Animals and Diets

Cows used in the present study were from the experiment of Khan et al. (2014) and were free of clinical disease throughout the study. Briefly, one group of cows was fed a diet providing at least 100% of calculated NE_L (**CON**; 1.34 Mcal/kg of DM), whereas the other group was fed with a diet providing >140% calculated NE_L (**HE**; 1.62 Mcal/kg of DM) during the entire 45-d dry period. Diets were fed as TMR once a day (0600 h) using an individual gate feeding system (American Calan, Northwood, NH). Cows were housed in a ventilated enclosed barn during the dry period. After parturition, all cows were moved to a tiestall barn and fed a common lactation diet (NE_L = 1.69 Mcal/kg of DM) as TMR daily (0600 h) and milked twice daily (0400 and 1600 h).

PMNL Isolation

Samples of blood (~120 mL) were collected at ~0700 h from the coccygeal vein or artery with evacuated tubes containing acid citrate dextrose (ACD Solution A; Fisher Scientific, Pittsburgh, PA) at −14 ± 2, 7, and 14 d relative to parturition. After blood collection, tubes were placed on ice (~30 min) until isolation (Moyes et al., 2009). Samples were centrifuged at 600 × *g* for 15 min at 4°C. The buffy coat and approximately one-fourth of the red blood cells were removed and discarded. The remaining sample was poured into a 50-mL tube. Twenty milliliters of deionized water at 4°C was added to lyse red blood cells followed by addition of 5 mL of 5× PBS at 4°C to restore an iso-osmotic environment. Samples were then centrifuged at 200 × *g* for 10 min at 4°C. Subsequently, samples were washed with 1× PBS and re-collected by centrifugation at 500 × *g* for 3 min at 4°C three times. The isolated PMNL were immediately homogenized in 2 mL of TRIzol reagent (Invitrogen, Carlsbad, CA) with 1 μL of linear acrylamide (Ambion Inc., Austin, TX) using a Polytron power homogenizer (Kinematica, Bohemia, NY) at maximum speed. The suspension was then transferred equally into 2 RNA-free microcentrifuge tubes (2 mL; Fisher Scientific) and stored at −80°C until further analysis.

RNA Isolation

The suspension of RNA and TRIzol reagent was thawed and, upon centrifugation, total RNA was separated with chloroform followed by acid phenol:chloroform (Ambion Inc.). Total RNA was then precipitated with isopropanol, and the RNA pellet was cleaned with 75% ethanol before reconstitution in RNA storage buffer (Ambion Inc.) for storage at −80°C. Purity of RNA was confirmed by optical density $(OD)_{260nm}/OD_{280nm}$ (NanoDrop ND-1000, NanoDrop Technologies, Rockland, DE). Quality of RNA evaluated via RNA integrity number (RIN) in the 2100 Bioanalyzer (Agilent Technologies Inc., Santa Clara, CA) was 7.7 ± 0.2.

Quantitative PCR Analysis

All procedures were as reported previously (Moyes et al., 2014). Briefly, cDNA was synthesized using 100 ng of RNA, 1 μg of dT18 (Operon Biotechnologies, Huntsville, AL), 1 μL of 10 mmol/L dNTP mix (Invitrogen Corp., Carlsbad, CA), 1 μL of random primers (3 mg/μL; Invitrogen Corp.), and 10 μL of DNase-/RNase-free water. The mixture was incubated at 65°C for 5 min and kept on ice for 3 min. A total of 6 μL of master mix, composed of 5.5 μL of 5× reaction buffer, 0.25 μL