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Maternal supply of methionine during late pregnancy is associated with changes in immune function and abundance of microRNA and mRNA in Holstein calf polymorphonuclear leukocytes

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ABSTRACT

Pregnancy and early life are critical periods during which environmental factors such as nutrition can affect development. Rumen-protected methionine (Met; RPM) supplementation during the prepartum period improves not only performance but immune responses in dairy cows. We investigated the effects of enhanced maternal supply of Met via feeding RPM on whole-blood in vitro lipopolysaccharide (LPS; 0, 0.01, or 5 μg/mL of blood) challenge and targeted microRNA and mRNA abundance in calf blood polymorphonuclear leukocytes (PMNL). Calves (n = 12/maternal diet) born to cows fed RPM at 0.08% of diet dry matter (DM)/d (MET) for the last 21 ± 2 d before calving or fed a control diet with no added Met (CON) were used. The PMNL were isolated at birth (before colostrum feeding) and d 1 (24 h after colostrum intake), 14, 28, and 50 of age. Maternal blood was collected at −10 ± 1.3 d relative to calving. Cows in the MET group had greater DM intake and lower prepartal haptoglobin concentration. In CON cows, haptoglobin was positively correlated with proinflammatory and host-defense mRNA abundance in CON calves. Except for *NOS2* and *NFE2L2*, abundance of *CASP8*, *MPO*, *ZBP1*, and *TNF* was lower at birth in MET calves. Interleukin 1β concentration in response to LPS challenge in CON and MET calves was greatest at birth, underscoring the role of this cytokine for lymphocyte activation. Compared with 1 d of age, the interleukin-1β response to incremental doses of LPS was greater at 14 through 28 d, suggesting that the neonatal calf can mount a robust response to inflammatory stimuli. Greater abundance in CON calves of *NOS2*, *CADM1*, and *TLR2* coupled with lower *SELL* from 1 through 50 d of age suggested a chronic activation of the PMNL. There was a marked upregulation over time of *MIR125b*, *MIR146a*, *MIR155*, and *MIR9* in both CON and MET calves, suggesting that these microRNA could affect gene transcription associated with differentiation and inflammatory function in PMNL. Regardless of maternal diet, the gradual downregulation of *MIR223* (the most abundant microRNA in PMNL) is in line with the progressive increase over time in the proinflammatory signature of the PMNL. Data revealed the potential for maternal supply of Met during late pregnancy through either greater DM intake or Met to elicit some changes in PMNL function during early postnatal life, partly through changes in mRNA expression encompassing cell adhesion and chemotaxis, oxidative stress, Toll-like receptor signaling, and Met metabolism.

Key words: glutathione, transsulfuration, inflammation, nutritional programming

INTRODUCTION

Enhancing the supply of Met via feeding with rumen-protected Met (**RPM**) during the periparturient period increases DMI, which is extremely important for reducing incidence of metabolic disorders around calving (Osorio et al., 2013; Roche et al., 2013). A reduction in proinflammatory status also has been observed in cows fed RPM during the peripartal period (Osorio et al., 2014; Zhou et al., 2016c). All these effects are associated with better milk production, reduced metabolic disorders (ketosis and retained placenta), and better overall liver function and innate immune response (Zhou et al., 2016a).

For calves, understanding the relationship between maternal and neonatal biomarkers of stress and inflammation during the neonatal period could help in devising measures to reduce inflammation and improve health during early life (Bertoni et al., 2009). At birth, calves are exposed to an initial stress that is compounded by additional stresses associated with common husbandry approaches such as weaning; hence, immune challenges

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early in life are an important component of the neonatal period (Ranade et al., 2014). Therefore, manipulation of maternal nutrition to improve health during the neonatal period and reduce the initial stress of birth is of importance (Jacometo et al., 2016).

There have been tremendous advances over the years in our knowledge of the nutritional physiology of the calf (Blum, 2006; Naeem et al., 2012). However, the extent to which prenatal maternal nutrition affects the molecular profiles of inflammatory pathways and polymorphonuclear leukocyte (**PMNL**) function in the neonatal animal is not well defined. Molecular tools clearly could enhance our understanding of how nutrition regulates basic functions of immune cells during stressful periods (Everts et al., 2005; Moyes et al., 2010). Methionine is one of the limiting AA for the newborn calf, and the supplementation of milk replacers with Met and Lys improved calf performance (Hill et al., 2008). Besides the role of AA in protein synthesis, there are data indicating a benefit of supplementing dietary AA that can affect the regulation of metabolic pathways to sustain the immune response against pathogens (Li et al., 2007).

Maternal circulating AA is reduced around calving (Zhou et al., 2016b), and the effect on calf immunometabolism still needs to be investigated. Recently, an in vitro study with neonatal Holstein calf PMNL demonstrated that the supplementation with Met, choline, or taurine increased homocysteine synthesis, mitigated the inflammatory response, and affected the antioxidant systems, highlighting the important role of these compounds in establishing an adequate immune response during the neonatal period (Abdelmegeid et al., 2017).

Currently, no reports on the effects of maternal supplementation of methyl donors during the dry period have evaluated residual effects on the transcription of genes related to immune function in the calf PMNL. Thus, the specific objective of this study was to evaluate the response to an in vitro whole-blood LPS challenge and profile the expression of 29 mRNA and 5 microRNA related to various biologic processes in PMNL using calves born to cows fed control or RPM diets during the last 21 ± 2 d of pregnancy. Results from the same study on blood biomarkers of metabolism, inflammation, and liver function and hepatic transcription of genes associated with metabolic processes and the 1-carbon metabolism have been reported previously (Jacometo et al., 2016, 2017).

MATERIALS AND METHODS

All procedures for this study were conducted in accordance with a protocol approved by the Institutional Animal Care and Use Committee of the University of Illinois (protocol no. 13023).

Maternal Treatments

Details of the experimental design have been published previously (Jacometo et al., 2016). Briefly, 40 multiparous Holstein cows received a common early dry period diet (far-off diet) from −50 to −22 d relative to parturition, with low energy and high straw designed to meet and not greatly exceed 100% of energy requirements. Cows received a higher energy diet from −21 ± 2 d until calving day (close-up diet). Calves were born to cows randomly assigned to receive RPM (Smartamine, Adisseo NA, Alpharetta, GA) at 0.08% of diet DM/d (**MET**; n = 20; ~2.9:1 Lys:Met) or no supplemental Met (**CON**; n = 20; ~3.35:1 Lys:Met) during the close-up period (Supplemental Table S1; https://doi.org/10.3168/jds.2018-14428). The Met supplement was top-dressed on the TMR. Ingredient and chemical composition of the diets is reported in Supplemental Table S1 (https://doi.org/10.3168/jds.2018-14428). Cow BW (773 ± 11 kg) and BCS (3.51 ± 0.05) did not differ. After birth, calves were fed a common diet and managed similarly.

Animal Management and Calf Enrollment Criteria

Complete details of these methods have been reported elsewhere (Jacometo et al., 2016). Briefly, during the dry period, cows were housed in a ventilated, sand-bedded freestall barn. Diets were fed for ad libitum intake as a TMR once daily using an individual gate feeding system (American Calan, Northwood, NH), and DMI was recorded daily. Colostrum volume was recorded and IgG content was estimated immediately based on specific gravity with a bovine colostrometer (cat. no. C10978N; Nasco, Fort Atkinson, WI). Calves were fed fresh colostrum from their respective dams within 6 h after birth. If voluntary colostrum intake had not reached the required 3.8 L, calves were force-fed via esophageal tube. Colostrum yield (7.6 kg), IgG content (78.7 mg/dL), total free AA concentration (301 μ/mL), and Met concentration (0.29% of total AA) did not differ (Jacometo et al., 2016, 2017).

Calf enrollment criteria, management after birth, and housing were described in detail elsewhere (Jacometo et al., 2016). Briefly, calves were housed in individual outdoor hutches bedded with straw, fed twice daily with a milk replacer (28.5% CP, 15% fat; Advance Excelerate, Milk Specialties, Carpentersville, IL; 520 g/d from 1 to 10 d of age, 680 g/d from 11 to 20 d of age, 840 g/d from 21 to 35 d of age, and 420 g/d from 36 to 42 d of age in a single feeding), and had ad libitum access to a