J. Dairy Sci. 98:1–13
http://dx.doi.org/10.3168/jds.2015-9359

Maternal consumption of organic trace minerals (4-Plex) alters calf systemic and neutrophil mRNA and microRNA indicators of inflammation and oxidative stress

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ABSTRACT

Organic trace mineral (ORG) supplementation to dairy cows in substitution of sulfate (INO) sources has been associated with improvement in immune function during stressful states such as the peripartal period. However, the effect of supplemental ORG during pregnancy on the neonatal calf is unknown. Therefore, our aim was to investigate the effects of ORG supplementation during late pregnancy on the immune system and growth of the neonatal calf. Of specific interest was the evaluation of inflammation-related microRNA (miRNA) and target gene expression in blood neutrophils as indicators of possible nutritional programming. Forty multiparous cows were supplemented for 30 d prepartum with 40 mg/kg of Zn, 20 mg/kg of Mn, 5 mg/kg of Cu, and 1 mg/kg of Co from either organic (ORG) or sulfate (INO) sources (total diet contained supplemental 75 mg/kg of Zn, 65 mg/kg of Mn, 11 mg/kg of Cu, and 1 mg/kg of Co, and additional Zn, Mn, and Co provided by sulfates), and a subset of calves (n = 8/treatment) was used for blood immunometabolic marker and polymorphonuclear leukocyte (PMNL) gene and miRNA expression analyses. Samples were collected at birth (before colostrum feeding), 1 d (24 h after colostrum intake), and 7 and 21 d of age. Data were analyzed as a factorial design with the PROC MIXED procedure of SAS. No differences were detected in BW, but maternal ORG tended to increase calf withers height. Calves from INO-fed cows had greater concentrations of blood glucose, GOT, paraoxonase, myeloperoxidase, and reactive oxygen metabolites. Antioxidant capacity also was greater in INO calves. The PMNL expression of toll-like receptor pathway genes indicated a pro-inflammatory state in INO calves, with greater expression of the inflammatory mediators *MYD88*, *IRAK1*, *TRAF6*, *NFKB*, and *NFKBIA*. The lower expression of miR-155 and miR-125b in ORG calves indicated the potential for maternal organic trace minerals in regulating the PMNL inflammatory response at least via alterations in mRNA and miRNA expression. Overall, these results indicate that maternal nutrition with organic trace minerals could alter the neonatal innate immune response at least in part via changes in gene and miRNA expression. Further studies involving inflammatory challenges during the neonatal period should be performed to determine the functional benefit of maternal organic trace minerals on the neonatal immune response.

Key words: epigenetics, fetal programming, nutrition, transcriptomics

INTRODUCTION

Trace mineral elements such as Cu, Cr, and Zn have important roles in the health and immunity of peripartal dairy cows (Spears and Weiss, 2008). Minerals have been commonly supplemented to cattle in the form of inorganic salts, preferably as sulfates; however, the development of organic forms of trace minerals, such as minerals complexed with AA, minimize the risk of mineral antagonism and enhance absorption efficiency (Swecker, 2014). Compared with a sulfate source, supplementing lactating cows with organic Zn resulted in greater immune response as well as improved milk yield (Wang et al., 2013).

The implications of trace mineral deficiency or impaired placental transfer of these minerals to fetal and neonatal ruminant metabolism have been studied for more than 30 yr (Hidiroglou, 1980). For instance, dairy calves supplemented with an injectable trace mineral

Received January 19, 2015.
Accepted July 8, 2015.
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complex containing Se, Cu, Zn, and Mn experienced an increase in neutrophil (PMNL) and glutathione peroxidase activity, and a reduction in the incidence of diarrhea, pneumonia, and otitis (Teixeira et al., 2014). These constitute an example of the innate immune response of the animal, one in which cells such as PMNL are partly regulated via signaling pathways and changes in mRNA expression. An important inflammation-responsive pathway in the animal that connects the innate and adaptive immune response is the toll-like receptor (**TLR**). Its activation, and that of its target genes, leads to stimulation of several intermediate molecules and culminates with the synthesis of classical pro-inflammatory cytokines and chemokines (Wolowczuk et al., 2008; Chen et al., 2015).

Although changes in mRNA expression are known to partly control adaptations in PMNL due to inflammation, more recent studies have concluded that epigenetic modifications through microRNA (**miRNA**) are an important part of the regulation of several cellular process (Aguilera et al., 2010) that modulate PMNL function including regulation of senescence, differentiation, adherence capacity, and cytokine production (Gantier, 2013). These observations are of interest in the context of dairy cow nutrition because epigenetic markers are candidates for bearing the memory of specific intrauterine nutritional exposure causing alterations in long-term gene expression, and consequently inducing developmental adaptations in physiology and metabolism (Attig et al., 2010).

Mature miRNA are non-protein-coding small RNA (~20 nucleotides length) that repress translational activity, promote destabilization of target mRNA, and regulate the abundance of mRNA target genes; however, these mechanisms remain under debate (Eulalio et al., 2008). It was reported in vitro using mouse P19 embryonal carcinoma cells that miR125-b can regulate mammalian neuronal differentiation by downregulating both translational efficiency and mRNA abundance of *lin-28* (Wu and Belasco, 2005). Using a microarray approach with miR-transfected HeLa cells (miR-1 and miR-124), it was demonstrated that miRNA could reduce the levels of many of their target transcripts, not just the amount of protein derived from these transcripts (Lim et al., 2005).

The general hypothesis of the present study was that maternal supplementation with organic trace minerals would improve neonatal calf metabolism and immune function reflected in the profiles of systemic blood biomarkers, mRNA, and microRNA in PMNL, and measures of growth and performance from birth through weaning. The possible epigenetic regulation through the action of miRNA in the pro-inflammatory signaling pathway also was evaluated.

MATERIALS AND METHODS

All the procedures for this study were conducted in accordance with the protocol approved by the Institutional Animal Care and Use Committee of the University of Illinois (protocol #12097).

Maternal Treatments

The experiment was conducted as a randomized complete blocked design with 40 multiparous Holstein cows blocked according to parity, previous lactation milk yield, and expected day of parturition. All cows received a common lactation diet (1.76 Mcal/kg of DM and 16.7% CP) during the last 60 d of lactation before dry-off, and a common early-dry period diet (1.1 Mcal/kg of DM, 14.5% CP) from −50 to −30 d relative to parturition. Both diets were supplemented at 100% of NRC (NRC, 2001) requirements with Zn, Mn, Cu, and Co in the form of an inorganic trace mineral mix. All cows received the same diet (1.5 Mcal/kg of DM, 15% CP) from −30 d to parturition (close-up period).

The close-up diet was partially supplemented with an inorganic trace mineral mix of Zn, Mn, and Cu to supply 35, 45, and 6 mg/kg, respectively, of the total dietary minerals. Cows were randomly assigned to an oral administration of a bolus once daily at the time of feeding the TMR. This contained a mix of either inorganic (**INO**, n = 20) or organic (AvailaZn Zn AA complex, AvailaMn Mn AA complex, AvailaCu Cu AA complex, and CoPro cobalt glucoheptonate; Zinpro Corporation, Eden Prairie, MN; **ORG**, n = 20) Zn, Mn, Cu, and Co to achieve 75, 65, 11, and 1 mg/kg, respectively, in diet DMI. After birth, calves were fed a common diet and managed similarly. Hence, any observed treatment effects are attributed to maternal nutrition during the last 30 d of gestation.

Animal Management and Calf Enrollment Criteria

During the dry period, cows were housed in a ventilated, sand-bedded free-stall barn, with a photoperiod of 8 h of light and 16 h of dark. Diets were fed for ad libitum intake as a TMR once daily, between 0600 and 0800 h, using an individual gate feeding system (American Calan, Northwood, NH). As cows began demonstrating signs of impending parturition, they were moved to an individual maternity pen bedded with straw. After parturition, cows were moved within 2 h to an individual chute and then milked with a porta-milker vacuum pump (catalog no. Z15664N, Nasco, Fort Atkinson, WI). Colostrum volume was recorded and IgG content was estimated based on specific gravity with a bovine colostrometer (catalog no. C10978N, Nasco).