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Maternal rumen-protected methionine supplementation and its effect on blood and liver biomarkers of energy metabolism, inflammation, and oxidative stress in neonatal Holstein calves

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ABSTRACT

In nonruminants, nutrition during pregnancy can program offspring development, metabolism, and health in later life. Rumen-protected Met (RPM) supplementation during the prepartum period improves liver function and immune response in dairy cows. Our aim was to investigate the effects of RPM during late pregnancy on blood biomarkers (23 targets) and the liver transcriptome (24 genes) in neonatal calves from cows fed RPM at 0.08% of diet dry matter/d (MET) for the last 21 d before calving or controls (CON). Blood (n = 12 calves per diet) was collected at birth before receiving colostrum (baseline), 24 h after receiving colostrum, 14, 28, and 50 d (post-weaning) of age. Liver was sampled (n = 8 calves per diet) via biopsy on d 4, 14, 28, and 50 of age. Growth and health were not affected by maternal diet. The MET calves had greater overall plasma insulin concentration and lower glucose and ratios of glucose-to-insulin and fatty acids-to-insulin, indicating greater systemic insulin sensitivity. Lower concentration of reactive oxygen metabolites at 14 d of age along with a tendency for lower overall concentration of ceruloplasmin in MET calves indicated a lesser degree of stress. Greater expression on d 4 of fructose-bisphosphatase 1 (*FBP1*), phosphoenolpyruvate carboxykinase 1 (*PCK1*), and the facilitated bidirectional glucose transporter *SLC2A2* in MET calves indicated alterations in gluconeogenesis and glucose uptake and release. The data agree with the greater expression of the glucocorticoid receptor (*GR*). Greater expression on d 4 of the insulin receptor (*INSR*) and insulin-responsive serine/threonine-protein kinase (*AKT2*) in MET calves indicated alterations in insulin signaling. In that context, the similar expression of sterol regulatory element-binding transcription factor 1 (*SREBF1*) in CON and MET during the preweaning period followed by the marked upregulation regardless of diet after weaning (d 50) support the idea of changes in hepatic insulin sensitivity during early postnatal life. Expression of carnitine palmitoyltransferase 1A (*CPT1A*) was overall greater and acyl-CoA oxidase 1 (*ACOX1*) was lower in MET calves, indicating alterations in fatty acid oxidation. Except forkhead box O1 (*FOXO1*), all genes changed in expression over time. Transcriptome results indicated that calves from MET-supplemented cows underwent a faster maturation of gluconeogenesis and fatty acid oxidation in the liver, which would be advantageous for adapting to the metabolic demands of extrauterine life.

Key words: nutritional programming, nutrition, methyl donors, transcriptomics

INTRODUCTION

During pregnancy, the maternal diet is one important factor that can elicit epigenetic effects in the offspring with long-term metabolic and physiologic consequences (Wu et al., 2004; Barua and Junaid, 2015). Epigenetic alterations can be induced through methylation of DNA and histones, acetylation of histones, or changes in microRNA expression, all of which alter transcription of the target genes (Jaenisch and Bird, 2003). In one of the first studies with ruminants, a low-protein diet fed to pregnant sheep resulted in lower methylation level of CpG islands in the fetal *IGFR2* (insulin-like growth factor 2 receptor) and *H19* genes in longissimus muscle, both of which have key roles in regulating growth and body composition (Lan et al., 2013). Limiting protein intake during pregnancy in rats also reduced

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histone acetylation of the liver-X-receptor α (*NR1H3*) promoter region in the offspring, silencing its expression and increasing the expression of its target glucose 6-phosphatase (*G6PC*), a key player in gluconeogenesis (Vo et al., 2013). Clearly, these data underscored the role of maternal protein nutrition on the programming of metabolic functions in the offspring.

More recent work in nonruminant species has revealed that maternal dietary methyl donors (e.g., Met, folic acid, betaine) play a role in nutritional programming of the offspring; that is, the concept that differences in nutritional experience at critical periods in early life, both pre- and postnatally, can program an individual's development, metabolism, and health for the future (Ji et al., 2016). In the context of gene transcription regulation, methyl donors serve as precursors of S-adenosylmethionine that could be used via methyltransferases to methylate DNA, RNA, and histones (Hollenbeck, 2012; Lin et al., 2014). In newborn piglets, it was demonstrated that maternal folic acid supplementation altered the expression of genes associated with immunity, oxidative stress response, and hepatic energy metabolism (Liu et al., 2013). In addition, supplementing betaine to sows during pregnancy resulted in alterations in the expression of gluconeogenic genes in the liver of newborn piglets partly through changes in DNA methylation (Cai et al., 2014). Whether similar responses occur in dairy calves is unknown, but recent work from our laboratory revealed that supplementation of pregnant cows with organic trace minerals from −30 d to calving (40 mg/kg of diet DM of Zn, 20 mg/kg of Mn, 5 mg/kg of Cu, and 1 mg/kg of Co) was associated with changes in expression of micro RNA in blood neutrophils and also systemic biomarkers of inflammation, oxidative stress, and liver function during the first 21-d of life (Jacometo et al., 2015).

Our general hypothesis was that rumen-protected Met supplementation during late-pregnancy, besides benefitting cows (Osorio et al., 2013, 2014a,b), would also be associated with changes in neonatal calf liver gene expression. Thus, the specific objectives of the present study were to determine the expression of genes related to energy metabolism, insulin signaling pathway, growth hormone-IGF-1 axis, glucocorticoid and adrenergic receptors, and also concentrations of immunometabolic biomarkers of metabolism, liver function, inflammation, and oxidative stress.

MATERIALS AND METHODS

All the 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 #13023).

Maternal Treatments

Calves in the present study were from cows randomly assigned to receive rumen-protected Met (**MET**, n = 21; Smartamine M, Adisseo NA, Alpharetta, GA) at 0.08% of diet DM/d (~2.8:1 Lys:Met) or no supplemental Met (**CON**; n = 20, ~3.6:1 Lys:Met) from −21 ± 2 before expected calving date until 30 DIM. The MET supplement was top-dressed once daily at the morning feeding using approximately 50 g of ground corn as carrier for all treatments. The TMR DM for the close-up and lactation diets was measured weekly for estimation of daily TMR DM offered. Supplementation of Met (0.08% DM of TMR offered) was calculated daily for each cow. Smartamine M was supplied as small beads containing a minimum of 75% DL-Met, physically protected by a pH-sensitive coating, which is considered to have a Met bioavailability of 80% (Graulet et al., 2005); therefore, per 10 g of Smartamine M, the cows received 6 g of metabolizable Met. The Met supplement was top-dressed on the TMR. Ingredient and chemical composition of the diets is in the Supplemental Table S1 (http://dx.doi.org/10.3168/jds.2016-11018). Cow BW (773 ± 11 kg) and BCS (3.51 ± 0.05) did not differ. After birth, calves were fed the same milk replacer and starter, and managed similarly.

Animal Management and Calf Enrolment Criteria

During the dry period, cows were housed in a ventilated, sand-bedded freestall 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 using an individual gate feeding system (American Calan, Northwood, NH) and DMI was recorded daily. As cows began demonstrating signs of impending parturition, they were moved to an individual maternity pen bedded with straw. On average, cows remained in the maternity pen for 3.69 ± 3.61 d. After parturition, cows were milked at the end of the farm's next milking period (0400, 1200, or 2000 h). Colostrum volume was recorded and IgG content was estimated based on specific gravity with a bovine colostrometer (Nasco, Fort Atkinson, WI; Cat. no. C10978N).

Calves were kept in the experiment if they fulfilled all the following criteria: (1) single calf; (2) calving difficulty score <3; (3) dam's colostrum quality assessed by a bovine colostrometer of >50 mg/L of IgG; (4) dam produced at least 3.8 L of a good-quality first