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Rumen-protected methionine compared with rumen-protected choline improves immunometabolic status in dairy cows during the peripartal period

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ABSTRACT

The immunometabolic status of peripartal cows is altered due to changes in liver function, inflammation, and oxidative stress. Nutritional management during this physiological state can affect the biological components of immunometabolism. The objectives of this study were to measure concentrations of biomarkers in plasma, liver tissue, and milk, and also polymorphonuclear leukocyte function to assess the immunometabolic status of cows supplemented with rumen-protected methionine (MET) or choline (CHOL). Forty-eight multiparous Holstein cows were used in a randomized complete block design with 2 × 2 factorial arrangement of MET (Smartamine M, Adisseo NA) and CHOL (ReaShure, Balchem Inc., New Hampton, NY) level (with or without). Treatments (12 cows each) were control (CON), no MET or CHOL; CON and MET (SMA); CON and CHOL (REA); and CON and MET and CHOL (MIX). From −50 to −21 d before expected calving, all cows received the same diet [1.40 Mcal of net energy for lactation (NE_L)/kg of DM] with no MET or CHOL. From −21 d to calving, cows received the same close-up diet (1.52 Mcal of NE_L/kg of DM) and were assigned randomly to each treatment. From calving to 30 d, cows were on the same postpartal diet (1.71 Mcal of NE_L/kg of DM) and continued to receive the same treatments until 30 d. The MET supplementation was adjusted daily at 0.08% DM of diet, and CHOL was supplemented at 60 g/cow per day. Liver (−10, 7, 21, and 30 d) and blood (−10, 4, 8, 20, and 30 d) samples were harvested for biomarker analyses. Neutrophil and monocyte phagocytosis and oxidative burst were assessed at d 1, 4, 14, and 28 d. The MET-supplemented cows tended to have greater plasma paraoxonase. Greater plasma albumin and IL-6 as well as a tendency for lower haptoglobin were detected in MET- but not CHOL-supplemented cows. Similarly, cows fed MET compared with CHOL had greater concentrations of total and reduced glutathione (a potent intracellular antioxidant) in liver tissue. Upon a pathogen challenge in vitro, blood polymorphonuclear leukocyte phagocytosis capacity and oxidative burst activity were greater in MET-supplemented cows. Overall, liver and blood biomarker analyses revealed favorable changes in liver function, inflammation status, and immune response in MET-supplemented cows.

Key words: inflammation, oxidative stress, transition cow, nutrition

INTRODUCTION

During the peripartal period, dairy cattle experience a state of negative energy and MP balance due to reduced DMI and increased nutrient requirements to support fetal growth and lactation (Drackley, 1999; Bell et al., 2000). Although some cows are able to adapt physiologically without being afflicted with metabolic and infectious diseases, the metabolic and immunologic challenges that occur during the peripartal period are important factors that limit the ability of most cows to achieve optimal performance and immunometabolic status (Drackley, 1999; Loor et al., 2013).

Due to extensive microbial degradation in the rumen, dietary availability of key methyl donors [(e.g., methionine (**MET**) and choline (**CHOL**)] are limited (Sharma and Erdman, 1989; Girard and Matte, 2005). Therefore, a negative methyl donor balance also may be an important challenge for the peripartal dairy cow owing to the fact that the synthesis of key compounds such as phosphatidylcholine (**PC**) and carnitine in tissues requires methyl donors (Pinotti et al., 2002). The biological role of methyl donors goes beyond metabolism because they are important sources of the intracellular

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antioxidants glutathione (**GSH**) and taurine (Brosnan and Brosnan, 2006).

In the context of liver metabolism and function, endogenous synthesis of PC from supplemental MET or CHOL could play a role in the ability of the tissue to handle the incoming fatty acids produced from lipolysis of adipose tissue. It is well-established that excessive hepatic fatty acid infiltration (Drackley, 1999) can negatively affect the normal functions of the liver. In addition to hepatic fat infiltration, the peripartal period is characterized by an increase of reactive oxygen metabolite (**ROM**) production, the accumulation of which could deplete intracellular antioxidants such as GSH and give rise to oxidative stress that may cause substantial tissue damage (Bertoni et al., 2009; Sordillo et al., 2009; Trevisi et al., 2012). Increased oxidative stress likely leads to inflammation and could compromise the leukocyte responses during the peripartal period.

Biomarkers in plasma, such as cholesterol and paraoxonase (**PON**), among others, have been used successfully to assess the degree of liver function around parturition (Bionaz et al., 2007; Bertoni et al., 2008; Loor et al., 2013). Furthermore, because parturition also is characterized by inflammatory conditions (Bionaz et al., 2007), proinflammatory cytokines (e.g., IL-1β; Trevisi et al., 2015) and the changes in concentrations of positive (e.g., haptoglobin) and negative acute-phase proteins (**APP**) offer a valuable tool to evaluate the functional welfare of the cow (Loor et al., 2013). Changes in inflammatory cytokines are closely linked with PMNL development and immunity-related activities (Burton et al., 2005); thus, coupling plasma biomarkers with measures of leukocyte function provide a more holistic view of the cow immune system. Immune dysfunction is a feature of the transition period and is characterized by impaired neutrophil trafficking, phagocytosis, and killing capacity (Kehrli et al., 1989; Goff and Horst, 1997), but also different ability of leukocytes to produce cytokines (Jahan et al., 2015). In the context of transition period management, the available data provide benchmarks that could be used to assess relationships among liver function, performance, and fertility (Bertoni and Trevisi, 2013).

Milk from dairy cows is high in methylated compounds and the levels secreted into milk are maintained even at the cost of depleting liver tissue reserves (Pinotti et al., 2002). Such an effect would exert an even greater challenge on the cow soon after calving, and coupled with the needs of cells to synthesize sulfur-containing antioxidants the supplementation of MET (and potentially CHOL) may be beneficial. However, to date, data demonstrating whether CHOL alone or in combination with MET provide equal or different benefits to the immunometabolic status in transition cows are limited.

Our general hypothesis was that supplementation of rumen-protected MET or CHOL improves liver function and alleviates inflammation and oxidative stress during the peripartal period. Therefore, the objectives of the present study were to measure concentrations of biomarkers in plasma, liver tissue, and milk, as well as PMNL function to assess the immunometabolic status of cows supplemented with MET or CHOL.

MATERIALS AND METHODS

Experimental Design and Dietary Treatments

All procedures for this study (protocol no. 13023) were approved by the Institutional Animal Care and Use Committee of the University of Illinois. Details of the experimental design have been described previously (Zhou et al., 2016b). Briefly, the experiment was conducted as a randomized complete block design with 2 × 2 factorial arrangement of MET (Smartamine M, Adisseo NA) and CHOL (ReaShure, Balchem Inc., New Hampton, NY) level (with or without). Cows were blocked according to expected calving date. Each block had 12 cows (except for the last block). Cows within each block were balanced for parity, previous lactation milk yield, and BCS before the close-up treatments were assigned. A total of 81 cows were used in a randomized, complete, unbalanced block design with 2 × 2 factorial arrangement of MET and CHOL level (with or without). Treatments were control (**CON**, n = 20), with no MET or CHOL supplementation; Smartamine (**SMA**, n = 21), CON plus MET at a rate of 0.08% of DM; Reashure (**REA**, n = 20), CON plus CHOL at 60 g/d; or Smartamine and Reashure (**MIX**, n = 20), CON plus MET plus CHOL. Dosage of MET was based on Osorio et al. (2013), whereas CHOL was supplemented following the manufacturer's recommendations. Per Institutional Animal Care and Use Committee guidelines, a subset of 48 multiparous cows (12 cows/treatment) were used for this portion of the study. All cows received the same far-off diet (1.40 Mcal/kg of DM, 10.2% RDP, and 4.1% RUP) from −50 to −22 d before expected calving, close-up diet (1.52 Mcal/kg of DM, 9.1% RDP, and 5.4% RUP) from −21d to expected calving, and lactation diet from calving (1.71 Mcal/kg of DM, 9.7% RDP, and 7.5% RUP) through 30 DIM.

The MET and CHOL supplements were both top-dressed from −21 ± 2 to 30 DIM once daily at the morning feeding using approximately 50 g of ground corn as carrier for all treatments. Supplementation of SMA (0.08% DM of TMR offered) was calculated daily