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Ethyl-cellulose rumen-protected methionine alleviates inflammation and oxidative stress and improves neutrophil function during the periparturient period and early lactation in Holstein dairy cows

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ABSTRACT

The periparturient period is the most critical phase in the productive cycle of dairy cows and is characterized by impairment of the immune system. Our objective was to evaluate the effect of feeding ethyl-cellulose rumen-protected methionine (RPM) starting at d −28 from expected parturition through 60 d in milk on biomarkers of inflammation, oxidative stress, and liver function as well as leukocyte function. Sixty multiparous Holstein cows were used in a block design and assigned to either a control or the control plus ethyl-cellulose RPM (Mepron, Evonik Nutrition & Care GmbH). Mepron was supplied from −28 to 60 d in milk at a rate of 0.09% and 0.10% dry matter during the prepartum and postpartum period. That rate ensured that the ratio of Lys to Met in the metabolizable protein was close to 2.8:1. Blood samples from 15 clinically healthy cows per treatment were collected at d −30, −14, 1, 7, 21, 30, and 60 and analyzed for biomarkers of liver function, inflammation, and oxidative stress. Neutrophil and monocyte function in whole blood was measured in vitro at −14, 1, 7, 21, and 30 d in milk. The statistical model included the random effect of block and fixed effect of treatment, time, and its interaction. Compared with control, ethyl-cellulose RPM increased plasma cholesterol and paraoxonase after parturition. Among the inflammation biomarkers measured, ethyl-cellulose RPM led to greater albumin (negative acute-phase protein) and lower haptoglobin than control cows. Although concentration of IL-1β was not affected by treatments, greater IL-6 concentration was detected in response to ethyl-cellulose RPM. Cows supplemented with ethyl-cellulose RPM had greater plasma concentration of ferric-reducing antioxidant power, β-carotene, tocopherol, and total and reduced glutathione, whereas reactive oxygen metabolites were lower compared with control cows. Compared with control, ethyl-cellulose RPM enhanced neutrophil phagocytosis and oxidative burst. Overall, the results indicate that ethyl-cellulose RPM supply to obtain a Lys-to-Met ratio of 2.8:1 in the metabolizable protein during the periparturient period and early lactation is an effective approach to help mitigate oxidative stress and inflammation as well as enhance liver and neutrophil function in dairy cows.
Key words: immunometabolism, methionine, transition period

INTRODUCTION

The periparturient period is the most critical phase in the productive life of high-producing dairy cows, and is characterized by the highest incidence and severity of both metabolic and infectious diseases (Bertoni et al., 2008; Trevisi et al., 2012; Sordillo, 2016). The inflammatory response can be activated by infection or injury, as well as tissue stress and malfunction in the absence of infection or evident tissue damage (Chovatiya and Medzhitov, 2014; Trevisi et al., 2016). The classic initiators of inflammation, infection and tissue injury, trigger the recruitment of leukocytes to the damaged or contaminated tissue, and immune cell function is fundamental for the efficiency and duration of the inflammatory process (Medzhitov, 2008). In cases of tissue stress or malfunction, the inflammatory response is mainly dependent on tissue-resident macrophages and, based on human disease models, it is responsible

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for chronic inflammatory conditions (Medzhitov, 2008). The inflammatory process triggers the acute-phase response, which alters liver function at least in part by switching to synthesis of positive acute-phase proteins (e.g., ceruloplasmin, haptoglobin) instead of negative acute-phase proteins (e.g., albumin) in an attempt to reestablish homeostasis (Cray et al., 2009).

Most dairy cows in early lactation experience a prolonged imbalance between production of free radicals and their elimination by antioxidants (Sordillo and Aitken, 2009), often causing cellular damage followed by a chronic inflammatory response. Oxidative stress around calving also can be a consequence of an infection-induced inflammatory response (Trevisi et al., 2016). Thus, the inflammatory events that afflict cows during early lactation are associated with physiological stressful conditions and the metabolic overload of the liver to meet the nutrient requirements for maintenance and milk synthesis (Trevisi et al., 2016).

It is well established that transition cows experience negative energy and protein balance (Drackley, 1999), and that nutrition plays a critical role in supporting the immune system (Bertoni et al., 2015; Sordillo, 2016). Methionine is the first-limiting AA in dairy cows (NRC, 2001), and recent data underscored its immunometabolic potential (Osorio et al., 2014; Sun et al., 2016; Zhou et al., 2016a). In the liver, methionine can be metabolized to S-adenosyl methionine, a fundamental methyl donor, as well as phosphatidylcholine, which is a main constituent of very low density lipoproteins. Furthermore, methionine metabolism can generate intracellular antioxidants such as glutathione (**GSH**) and taurine (Brosnan and Brosnan, 2006). Therefore, our general hypothesis was that feeding ethyl-cellulose rumen-protected methionine (**RPM**) to obtain a Lys-to-Met ratio of 2.8:1 in the MP from d −28 relative to the expected parturition to 60 DIM would help reduce the inflammatory and oxidative stress status and enhance liver function and leukocyte function in the cow.

MATERIALS AND METHODS

Animal Housing and Care

The Institutional Animal Care and Use Committee at the University of Illinois (Urbana; protocol #14270) approved all experimental procedures. All cows were housed in a freestall system equipped with the Calan gate system (American Calan Inc., Northwood, NH) during the prepartum period. After parturition, all cows were housed in tiestalls. Cows were fed once daily (1300 h) at 120% of expected intake, and milked 3 times daily (0600, 1400, and 2200 h).

Design and Treatments

Additional details of the experimental design have been described previously (Batistel et al., 2017). Briefly, 60 multiparous Holstein cows from the University of Illinois Dairy Research Farm were used in a randomized, complete, unbalanced block design experiment with 30 cows per treatment. The data presented herein is from a subset of cows. Cows were blocked by the expected parturition day, and the blocks were balanced by parity, previous 305-d milk yield, and BCS. The BCS used to block cows was measured at −30 d before parturition using the scale 1 to 5 (1 = thin, 5 = fat; scale in 0.25 increments). Cows within each block were randomly assigned to 1 of the 2 treatments. Treatment diets were a control diet without Met supplementation or the control diet plus ethyl-cellulose RPM (Mepron, Evonik Nutrition & Care GmbH). Ethyl-cellulose RPM was supplied from −28 to 60 d relative to parturition at a rate of 0.09 and 0.10% of the DMI of the previous day during the prepartum and postpartum period. Mepron is a commercial rumen-protected source of DL-Met that resists ruminal degradation through an ethyl-cellulose film coating. Pellets measure 1.8 × 3 mm and contain 85% DL-Met. The intestinal digestibility coefficient and rumen bypass of Mepron are 90 (Schwab, 1995) and 80% (Overton et al., 1996), respectively; therefore, per 10 g of Mepron, the cows received 6.1 g of Met available for absorption. The target values of Met supply were based on recent experiments demonstrating a benefit in terms of production performance and health of supplementing rumen-protected Met to achieve a Lys-to-Met ratio close to 2.8:1 during the prepartum and postpartum periods (Osorio et al., 2013; Zhou et al., 2016b). The Lys-to-Met ratio in the control group was close to 3.7:1 during the prepartum and postpartum periods. During the far-off period (from −45 to −29 d), all cows received the same diet (1.33 Mcal/kg of DM and 13.9% CP) with no RPM. The basal close-up (from −28 d to parturition), fresh (from 1 to 30 d), and high-production (from 31 to 60 d) diets contained 1.47 Mcal/kg of DM and 15.3% CP, 1.67 Mcal/kg of DM and 17.7% CP, and 1.61 Mcal/kg of DM and 17.4% CP, respectively. The ingredient and nutrient composition of the diets fed are reported in Table 1 and 2. All diets were formulated to meet cow predicted requirements according to NRC (2001).

Blood and Liver Sample Collection and Biomarker Analysis

Blood was sampled from the coccygeal vessel before the feeding in 15 cows per treatment at d −30, −14, 1,