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Supplemental methionine, choline, or taurine alter in vitro gene network expression of polymorphonuclear leukocytes from neonatal Holstein calves

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ABSTRACT

Isolated PMNL from neonatal calves were used to evaluate the effect of Met, choline, and taurine supplementation on mRNA expression of genes related to the Met cycle and innate immunity. Five neonatal Holstein calves (3 wk old) were used for PMNL isolation and in vitro culture. The selected genes were related to the 1-carbon and Met cycles, cell signaling and cytokine mediators, inflammation, antimicrobial and killing mechanism associated genes, immune mediators, adhesion, and pathogen recognition. The results indicated that supplementation of Met, choline, and taurine increased homocysteine synthesis through upregulation of *SAHH*. Furthermore, the lower expression of *CXCR1*, *IL10*, *IL6*, *IRAK1*, *NFKB1*, *NR3C1*, *SELL*, *TLR4*, and *TNFA* indicated that all treatments mitigated the inflammatory activation of blood PMNL. As indicated by the modulation of *GCLC* and *GPX1*, choline and taurine supplementation also affected the antioxidant system. However, data indicate that oversupplementation could alter the inflammatory and oxidative status, suggesting the existence of cytotoxicity thresholds. Overall, multiple biological processes in calf PMNL related to inflammatory response and cytoprotection against oxidative stress were affected by Met, choline, and taurine supplementation. These data underscore an important role of these compounds in pre-weaning calf nutritional management.

Key words: immunonutrition, inflammation, methyl donor, amino acid

INTRODUCTION

Although dairy producers follow advanced strategies in the management of dairy calves, 1 in 10 US dairy heifers dies before weaning due to exposure to different stressors, lack of immunity, or both (Hulbert and Moisa, 2016). Immaturity of the neonatal immune system, especially, has been identified as the crucial cause of the high morbidity in newborn calves (Gruse et al., 2016). Within the first 14 d of life, calves are most susceptible to enteritis, diarrhea, septicemia, and pneumonia (Svensson et al., 2006; Windeyer et al., 2014). Furthermore, healthy calves are unable to reach the stability needed to complete the maturation of the innate immune response even by 90 d of age (Batista et al., 2015).

Maintaining host health is a vital function of the immune system, and PMNL have a fundamental role in this process. As the primary mediators of the innate immune response, thanks to their pathogen-killing capacity, they represent the first line of defense against pathogens (Paape et al., 2003; Kobayashi and DeLeo, 2009). Their recruitment is the end result of a carefully orchestrated and complex signal transduction events (Kobayashi and DeLeo, 2009; Futosi et al., 2013). For example, changes in mRNA expression, such as the activation of toll-like receptor and its target genes, stimulate synthesis of classical pro-inflammatory cytokines and chemokines (Wolowczuk et al., 2008; Jacometo et al., 2015; Chen et al., 2016).

Alongside the role of AA as building blocks for protein synthesis, dietary supplementation of AA guarantees normal immunocompetence through provision and synthesis of specific proteins (such as cytokines) and regulation of key metabolic pathways to sustain the immune response against infectious pathogens (Li et al., 2007). The AA themselves have a role as cell signaling molecules (Li et al., 2007). These functions expedite the role of AA in the regulation of key metabolic pathways for maintenance, growth, reproduction, and immunity (Wu, 2009). Specifically to calves, Hill et al. (2008) concluded that Lys and Met are limiting AA for the newborn animal; hence, it is crucial to supplement milk protein-based milk replacers with synthetic Lys and Met to improve calf performance. The dairy NRC (2001) does not report individual AA requirements for calves, likely because such data are scarce in the

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literature. However, most commercially available milk replacers often include synthetic Met (DL-Met), Lys, and choline (**CHO**; choline chloride), whereas taurine (**TAU**) is not directly supplemented in pure form.

Rumen-protected Met is effective in providing extra metabolizable Met to balance peripartal diets of Holstein cows, improving PMNL phagocytosis capacity, migration, development, and cellular antioxidant capacity (Osorio et al., 2013; Li et al., 2016). Taurine (a Met derivative) has a key role itself in the regulation of the innate immune response by reducing tissue damage induced by bacterial infection, and it is the most abundant free AA in leukocytes, particularly in PMNL (Ekremoğlu et al., 2007; Erdamar et al., 2007; Miao et al., 2012). In addition to its anti-inflammatory and antioxidant effects, TAU possesses potent antimicrobial properties, as it can increase PMNL phagocytic ability and respiratory burst activity (Chorazy et al., 2002; Ekremoğlu et al., 2007).

We previously reported a greater postpartal PMNL activity in Met-supplemented transition cows (Osorio et al., 2013; Zhou et al., 2016b). However, to our knowledge, no literature is available to precisely describe the effects of Met, CHO, and TAU on PMNL immune function in newborn calves. We hypothesized that Met, CHO, and TAU might partly regulate PMNL function through alterations in gene expression; hence, we determined the effect of Met, CHO, and TAU supplementation in vitro on the mRNA expression of genes related to the Met cycle, cell signaling, inflammation, antimicrobial mechanisms, immune mediators, and adhesion molecules in PMNL isolated from neonatal calves.

MATERIALS AND METHODS

Animal Handling

Animal handling and all procedures received approval from the University of Illinois Institutional Animal Care and Use Committee (protocol #14270). Five Holstein calves (3 wk age) from the University of Illinois dairy herd were selected for PMNL isolation. Previous to blood collection, calves were fed and managed according to established standard operating procedures. First-milking colostrum was fed within 2 h after birth, and calves were housed in individual outdoor hutches bedded with straw. Calves were fed twice daily (0700 and 1800 h) with a milk replacer (Advance Excelerate, Milk Specialties, Carpentersville, IL; 28.5% CP, 15% fat; supplemented with L-Lys and DL-Met) and had ad libitum access to a starter grain mix (19.9% CP, 13.5% NDF) at 0800 h. Until 10 d of age, calves received 2.27 kg (5.0 lb) of milk replacer at each feeding, and were then switched to 2.95 kg (6.5 lb) until the time of sampling (3 wk of age). Calves did not have any recorded health problems at sampling time.

Blood Sample Collection

Blood was collected at 3 wk of age from a jugular vein at 0700 h, before feeding, into Vacutainer tubes (BD Vacutainer, Becton Dickinson and Company, Franklin Lakes, NJ) containing 1.5 mL of ACD solution A (trisodium citrate, 22.0 g/L; citric acid, 8.0 g/L; and dextrose 24.5 g/L). Fifteen tubes were collected, for a total of 120 to 150 mL of blood per each calf (Jacometo et al., 2015). Tubes were inverted 10 times, immediately placed on ice, and transported to the laboratory for immediate isolation of PMNL. No more than 40 min passed from the beginning of sampling to start of isolation.

PMNL Isolation and Viability Analysis

The PMNL were isolated as previously described (Jacometo et al., 2015). The complete procedure can be found in the Supplemental Material (https://doi.org/10.3168/jds.2016-12025). Briefly, samples were centrifuged to separate the phases, then plasma, buffy coat, and approximately one-third of the red blood cells were removed and discarded. The remaining material was combined and red blood cells were lysed using deionized water at 4°C, followed by addition of 5 × PBS at 4°C to restore isotonicity. Cells were pelleted by centrifugation and the supernatant was discarded. The pellet was then washed with 1× PBS and a second lysis step with deionized water was performed. After repelleting, 2 subsequent washings using 1× PBS at 4°C were performed. The resulting neutrophil pellets from the 5 animals were then mixed to obtain a homogeneous pool, and washed again with 1× PBS. Before incubation, to assess purity and viability, an aliquot was stained with the granulocyte antibody CH138A, followed by a second antibody (phycoerythrin and propidium iodide), before the reading with a flow cytometer. The neutrophil pool had a purity greater than 90% and viability greater than 90%.

PMNL Incubation and Treatments

Cells from the isolated pool of PMNL were counted using an automated cell counter (MOXI Z Mini, Orflo Technologies, Ketchum, ID) and diluted to a final concentration of 6×10^6 cells/mL with warm (37°C), Met-free incubation medium (RPMI 1640, Gibco, Thermo Fisher, Waltham, MA) and 8% of fetal bovine serum (Sigma-Aldrich, St. Louis, MO), previously heat-inactivated. From the diluted pool, aliquots of 1 mL