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Corium molecular biomarkers reveal a beneficial effect on hoof transcriptomics in peripartal dairy cows supplemented with zinc, manganese, and copper from amino acid complexes and cobalt from cobalt glucoheptonate

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ABSTRACT

Supplying trace minerals in more bioavailable forms such as amino acid complexes (AAC) could help ameliorate the incidence of hoof disorders in peripartal dairy cows. The aim of this study was to evaluate the effects of supplementing metal AAC during the peripartal period on expression of 28 genes in corium tissue related to claw composition, oxidative stress, inflammation, chemotaxis, and transcriptional regulation. Forty-four multiparous Holstein cows received a common diet from −30 to 30 d relative to parturition and were assigned to receive an oral bolus containing either inorganic trace minerals (INO) or AAC (i.e., organic) Zn, Mn, Cu, and Co to achieve supplemental levels of 75, 65, 11, and 1 ppm, respectively, in the total diet dry matter. Inorganic trace minerals were provided in sulfate form, and AAC were supplied via Availa Zn, Availa Mn, Availa Cu, and COPRO (Zinpro Corp., Eden Prairie, MN). Locomotion score was recorded before enrollment and weekly throughout the experiment. Incidence of hoof health problems at 30 DIM was evaluated before a hoof biopsy in a subset of cows (INO = 10; AAC = 9). Locomotion score did not differ between treatments in the prepartum or postpartum period. The incidence of heel horn erosion was lower in AAC cows, but the incidence of sole ulcers did not differ. Downregulation of *KRT5*, *CTH*, *CALML5*, and *CYBB*, and upregulation of *BTD* in AAC cows indicated a decrease in the need for activation of cellular pathways to regenerate corium tissue and increase biotin availability in the sole claw. These molecular changes in the sole could have been triggered by the lower incidence of heel erosion in response to AAC. Among the genes associated with oxidative stress, the AAC cows had greater expression of *NFE2L2*, a transcription factor that regulates the antioxidant response, and the antioxidant enzyme *SOD1*. Among genes associated with inflammation, AAC cows had greater expression of *TLR4*, and lower expression of *TLR2*, *IL1B*, and *TNF* compared with INO cows. Supplementation with metal AAC during the peripartal period affected the expression of genes involved in composition, oxidative stress, and inflammation status in the corium. The hoof biopsy procedure used in the present study should be further perfected and implemented in future lameness research to expand our understanding of hoof biology in dairy cows.
Key words: organic minerals, dairy cattle, transition period, gene expression

INTRODUCTION

Lameness is of great interest to the dairy industry because of its economic impact and effect on animal welfare (Cha et al., 2010; Shearer et al., 2013). Important trace minerals such as Zn, Mn, and Cu are essential cofactors for multiple enzymes including those involved in keratin formation; therefore, these trace minerals have been associated with hoof health (Tomlinson et al., 2004). The low DMI of early postpartal dairy cows relative to the large requirements for milk production compromises supply of most nutrients including trace minerals. In addition, negative interactions among trace minerals and macrominerals and dietary factors pre and post absorption can further reduce trace mineral bioavailability (NRC, 2001). Therefore, the use of organic forms [e.g., complexed, covalently bonded to AA; amino acid complex (**AAC**)] of these trace minerals has been widely adopted by the dairy industry.

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We have successfully demonstrated the feasibility of performing a hoof biopsy in dry, nonpregnant dairy cows with the aim of isolating mRNA from corium tissue to perform gene expression analyses, minimizing adverse effects on locomotion score and overall hoof health (Osorio et al., 2012). The use of this procedure allows for the in vivo study of important biological processes in the corium tissue, including keratinization, inflammation, and oxidative stress.

Keratinization of the bovine hoof is a complex and dynamic process that responds to stimuli (e.g., mechanical damage, trace minerals imbalance, inflammation) to maintain the strength and integrity of the hoof capsule (Hendry et al., 1997; Tomlinson et al., 2004). Among several keratins we evaluated for keratinization, *KRT5* was the most important at a transcriptional level in immature keratinocytes (Osorio et al., 2012). Biotin is an important vitamin for keratinization and hoof integrity (Tomlinson et al., 2004), underscoring the importance of biotinidase (encoded by *BTD*), an enzyme responsible for the use and recycling of biotin.

Based on previous corium transcriptomic data between susceptible and non-susceptible claws from dry nonpregnant cows (Osorio et al., 2012), we hypothesized that genes associated with keratinization, such as *KRT5* and *BTD*, would be affected by the supply and source of trace minerals. Furthermore, we hypothesized that proinflammatory genes would be downregulated with AAC supplementation. The proinflammatory response can be initiated via different pathways, including the toll-like receptors (**TLR**; e.g., *TLR2* and *TLR4*), which are perhaps the most widely studied. Upon activation of the TLR pathway, the primary outcome is the synthesis of proinflammatory cytokines (e.g., tumor necrosis factor, **TNF**, IL-1β, and IL-6; De Nardo, 2015). Activation of proinflammatory cytokines can upregulate matrix metalloproteinases that have collagenase activity and impair the integrity of the suspensory apparatus of the hoof capsule (Dufour, 2015). The objective of this study was to evaluate the effects of supplementing AAC during the peripartal period on genes related to claw composition, oxidative stress, inflammation, chemotaxis, and transcriptional regulation.

MATERIALS AND METHODS

Experimental Design and Dietary Treatments

The Institutional Animal Care and Use Committee of the University of Illinois approved all protocols for this study (protocol no. 12097). The experiment was conducted as a randomized complete block design where 44 multiparous Holstein cows were blocked according to parity, previous lactation milk yield, and expected day of calving (Osorio et al., 2016). The complete description of the experimental design is reported elsewhere (Osorio et al., 2016). Briefly, cows received a common diet supplemented at 100% of NRC (2001) requirements with Zn, Mn, Cu, and Co in the form of inorganic trace minerals (**INO**) from 110 to 30 d before calving. From −30 d to calving, cows received a common prepartal diet (1.5 Mcal/kg of DM, 15% CP), and from calving to 30 DIM a common postpartal diet (1.76 Mcal/kg of DM, 18% CP). Both diets were partially supplemented with an INO mix of Zn, Mn, and Cu to supply 35, 45, and 6 ppm, respectively, of the total diet DM. Cows were assigned to treatments in a randomized completed block design, receiving a daily oral bolus with a mix of INO or AAC containing 40, 20, 5, and 1 ppm of Zn, Mn, Cu, and Co, respectively, to achieve 75, 65, 11, and 1 ppm, respectively, in the total diet DM. Inorganic trace minerals were provided in sulfate form and AAC were supplied as Availa Zn, Availa Mn, Availa Cu, and COPRO (Zinpro Corp., Eden Prairie, MN). As described in Osorio et al. (2016) and Batistel et al. (2016), some cows were removed during the experiment due to health complications; none of those cows were included in the present analysis. Per Institutional Animal Care and Use Committee guidelines, 12 cows per treatment were approved for liver biopsies, corium biopsies, blood neutrophil RNA isolation, and blood biomarker analysis. However, from these subsets, only 10 INO and 9 AAC cows had a corium biopsy plus a complete set of liver biopsies, neutrophil RNA, and corresponding blood samples; only these cows were used for the corium mRNA expression analysis.

Animal Management and Locomotion Score

Prior to calving, cows were fed individually once daily at 0630 h using an individual gate system (American Calan, Northwood, NH). Cows were housed on concrete floors in a ventilated enclosed barn during the dry period and had access to sand-bedded free stalls until 3 d before expected parturition, when they were moved to individual maternity pens bedded with straw until parturition. After parturition, cows were housed in a tie-stall barn, fed a common lactation diet once daily, and milked 3 times daily. At 30 DIM, cows were returned to the farm herd. Feed offered was adjusted daily to achieve 5 to 10% refusals.

A locomotion score was assigned by 2 individuals before cows were enrolled in the experiment, based on a scale described by Flower and Weary (2006; 1 = normal, symmetrical gait and flat back to 5 = lame, asymmetric gait, extremely arched back) to discrimi-