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The effects of feeding mixed tocopherol oil on whole-blood respiratory burst and neutrophil immunometabolic-related gene expression in lactating dairy cows

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ABSTRACT

The 4 major tocopherol isoforms differ in their biochemical reactivity and cellular effects due to basic chemical structural differences. Alpha-tocopherol has been well studied regarding effects on bovine polymorphonuclear leukocyte (PMN) function and its involvement in respiratory burst. However, no studies to date have identified the effects of supplementing a mixed tocopherol oil (Tmix) particularly enriched in non-α tocopherol isoforms (i.e., γ- and δ-isoforms) on fundamental immunometabolic changes in dairy cows. Therefore, the objectives of this study were to determine whether short-term feeding of vegetable oil–derived Tmix alters specific biomarkers of metabolism, whole-blood leukocyte populations, respiratory burst, immunometabolic-related gene expression of PMN, or gene expression of isolated PMN when challenged with lipopolysaccharides (LPS). Clinically healthy multiparous lactating Holstein cows (n = 12; 179 ± 17 d in milk, 40.65 ± 3.68 kg of milk yield) were fed Tmix (620 g/d) for 7 consecutive days. Jugular blood (EDTA anticoagulant) was collected from all cows on d 0 before treatment initiation and again on d 7 after Tmix feeding. Total stimulated respiratory burst activity (RBA) and leukocyte populations were assessed in whole blood, and tocopherol isoform concentrations, metabolites, and hormones were measured in plasma. For gene expression analysis, isolated PMN from cows before and after Tmix feeding were incubated with LPS at a final concentration of either 0.0 or 1.5 μg/mL. Feeding of Tmix for 7 d increased the concentrations of α- and γ-tocopherol. The Tmix did not alter plasma insulin but decreased cholesterol. The Tmix did not alter whole-blood RBA or the leukocyte populations. The LPS challenge increased the expression of proinflammatory genes *TNFA* and *IL6*. However, Tmix treatment did not alter the patterns of LPS-affected expression of genes (e.g., *TNFA*, *ITGB2*, *PPARA*, and *RXRA*) associated with the immune or metabolic response. In conclusion, short-term feeding of Tmix may have no negative effect on animal health as Tmix increased α- and γ-tocopherol concentrations in blood and did not impair whole-blood RBA or alter leukocyte populations. The data provide further support that the α- and γ-tocopherol isoforms do not interfere with normal immune or metabolic function.
Key words: Holstein cow, mid lactation, tocopherol, health, neutrophil

INTRODUCTION

Bovine mastitis (i.e., an inflammation of the mammary gland) is usually associated with the invasion of a bacterial pathogen (Gruet et al., 2001). During mastitis, circulating PMN are recruited to the infection site and play an essential role in controlling the duration and severity of the infection-recovery process (Paape et al., 2003; Lauzon et al., 2005). However, PMN can have detrimental effects on the mammary tissue during the inflammatory response. Reasons could be host cell mammary gland damage (mainly via lipid peroxidation) associated with the overproduction of proinflammatory cytokines [i.e., tumor necrosis factor (**TNF**) α; Persson et al., 1993] and reactive oxygen species (**ROS**; Sordillo et al., 2009). These proinflammatory factors are acknowledged to contribute significantly to collateral metabolic disorders such as ketosis and milk fever or inflammation in other organs (Blum et al., 2000; Wellnitz and Bruckmaier, 2012; Sordillo, 2016).

Nutritional supplements of vitamin E have been studied with regard to mitigating the negative consequences

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of the inflammatory response in cows and calves (Barrett et al., 1997; Higuchi et al., 2013; Singh et al., 2013). Potential mechanisms of the beneficial effects of vitamin E during inflammation include (1) helping to control lipid peroxidation of host tissues mainly associated with infiltrating PMN, (2) controlling lipid peroxidation–induced inflammation, and (3) protecting the integrity of the membrane structure of PMN via alleviating lipid peroxidation during inflammation (Chew, 1995). Alpha-tocopherol (i.e., the most biologically active isoform of vitamin E) has been studied for its ability to improve PMN chemotaxis (Luostarinen et al., 1991) and enhance the expression of plasminogen activator urokinase receptor gene (*PLAUR*; Pinotti et al., 2003). In addition, α-tocopherol has been studied as a means of improving phagocytosis of PMN in cattle (Hogan et al., 1992) and modulating the expression of proinflammatory genes in PMN by controlling lipid peroxidation (i.e., oxidative stress) responses (Sordillo et al., 2009). Most vitamin E research has been conducted during the transition period (Qu et al., 2013; Pilotto et al., 2016), a high-risk period for disease (Drackley et al., 2006) when most cows experience a natural depletion of vitamin E (i.e., α-tocopherol). This depletion can increase susceptibility to disease (Pinotti et al., 2003; Qu et al., 2013, 2014). Much less information is available on the mitigating effects of vitamin E during mid lactation. For the current study, dairy cows in mid lactation were used to characterize the host response to feeding tocopherols to reduce the potential cofounding complications of the high-risk transition period.

Together, the α-, β-, γ-, and δ-tocopherol isoforms constitute the naturally occurring mixed tocopherol matrix (Spears and Weiss, 2008; Weiss and St-Pierre, 2009; Politis et al., 2012). In dairy cattle, α-tocopherol, the major isoform used in diet supplementation, has been studied more than any other isoform. Furthermore, because so much emphasis has been placed on the biological attributes of α-tocopherol, a major role for the other isoforms has been related to their conversion to the α-isoform (Netscher et al., 2007). What is recognized now that was not apparent at the time when α-tocopherol was first added as a supplement to the diet is that these non-α isoforms have a more diverse biochemical pedigree with their own unique healthful properties distinct from those attributable to α-tocopherol. For example, γ-tocopherol is recognized as distinct from α-tocopherol as an anti-inflammatory intervention where γ-tocopherol possesses cyclooxygenase activity and has a higher affinity to trap lipophilic electrophiles, many of which are derived from superoxide anion and nitric oxide during an inflammatory event within the membrane (Groeger and Freeman, 2010). In addition, mixed tocopherol is a natural oil extracted from several plant sources such as soybean and could have a positive effect on organic dairy practices. However, the effect of feeding additional mixed tocopherol isoforms on animal health has not been fully elucidated. It is becoming clearer that γ- and δ-tocopherols have overall health benefits additive and complementary to α-tocopherol (Zempleni et al., 2013). Therefore, the objectives of this study were to investigate how short-term feeding of vegetable oil–derived mixed tocopherol oil (**Tmix**) supplement (with high levels of γ- and δ-isoforms) might alter specific biomarkers of metabolism, whole-blood leukocyte populations and respiratory burst, and PMN immunometabolic-related gene expression. The hypotheses are that Tmix (α-, β-, γ-, δ-tocopherol) will not detrimentally alter animal health as determined via biomarkers of metabolism, leukocyte population, and respiratory burst status and that Tmix may differentially affect the expression of proinflammatory genes in circulating PMN.

MATERIALS AND METHODS

All procedures involving the use of live animals were approved in accordance with the regulations and guidelines set forth by the USDA Beltsville Animal Care and Use Committee (no. 15-009).

Animals and Treatments

Twelve multiparous Holstein cows in mid lactation (179 ± 17 DIM; 40.65 ± 3.68 kg/d milk yield) were used for this study. Due to the tiestall space limitation, the experiment was conducted in 3 blocks of 4 cows each (randomly picked from a total of 12 cows). Cows did not display clinical signs of disease and had an average composite milk SCC <100,000 cells/mL before enrollment in the study. Within each block, cows (n = 4) were housed and fed in tiestalls (each with its own feed bunk), had free access to water, and were milked twice daily at 0600 and 1800 h. Cows were fed a TMR (22.7 kg ± 1.3 kg/d) ad libitum (Table 1) formulated to meet NRC (2001) requirements (NE_L = 1.5 Mcal/kg of DM; CP = 17.03% of DM) for lactating dairy cows averaging 90 DIM and producing 40 kg of milk/d with a BW and BCS of 598 kg and 3.0 (on a 5-point scale; Ferguson et al., 1994), respectively. Cows were fed twice per day at 0700 and 1400 h with the diet portions equally split between the 2 feedings (total of 22.7 ± 1.3 kg of TMR/d). An initial blood sample was collected on d 0 before the morning feeding to represent the basal diet without Tmix supplementation (absence: −Tmix). All cows then received their morning feed top-dressed with the Tmix (620 g/d) for 7 consecutive days. Blood sampling was repeated on d 7 after Tmix supplementa-