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Injectable trace minerals (selenium, copper, zinc, and manganese) alleviates inflammation and oxidative stress during an aflatoxin challenge in lactating multiparous Holstein cows

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ABSTRACT

Trace minerals are vital in the antioxidant response during oxidative stress; however, limited research is available on the effects of trace mineral supplementation during an aflatoxin (AF) challenge. The objective of the study was to determine the effects of 2 subcutaneous injections of 15 mg/mL of Cu, 5 mg/mL of Se, 60 mg/mL of Zn, and 10 mg/mL of Mn (Multimin 90, Multimin North America, Fort Collins, CO) given at 1 mL/90.7 kg of average body weight in response to an AF challenge. Fifty-eight Holstein cows [body weight (mean ± SD) = 734 ± 6 0kg; days in milk = 191 ± 93] were assigned to 1 of 3 treatments in a randomized complete block design. The experimental period (63 d) was divided into an adaptation phase (d 1–56) and a measurement phase (d 57–63). From d 57 to 59, cows received an AF challenge that consisted of 100 μg of aflatoxin B_1/kg of dietary dry matter intake (DMI) administered orally via balling gun. Treatments were saline injection and no AF challenge (NEG), saline injection and AF challenge (POS), and trace mineral injection and AF challenge (MM). Injections were performed subcutaneously on d 1 and 29. Milk was sampled 3 times daily from d 56 to 63, blood was sampled on d 0, 56, 60, and 63, and liver samples were taken on d 0 and 60. Two treatment orthogonal contrasts [CONT1 (NEG vs. POS) and CONT2 (POS vs. MM)] were made. Cows in NEG had lower AF excretion in milk and greater 3.5% fat-corrected milk (32.1 ± 1.37 kg/d) compared with cows in POS (28.6 ± 1.43 kg/d). Feed efficiencies (3.5% fat-corrected milk/DMI, energy-corrected milk/DMI, and milk/DMI) were greater for cows in NEG (1.42 ± 0.07, 1.46 ± 0.07, and 1.45 ± 0.07, respectively) than cows in POS (1.16 ± 0.08, 1.18 ± 0.08, and 1.22 ± 0.07, respectively). Cows in POS had greater milk urea nitrogen and blood urea nitrogen than cows in MM. Liver concentrations of Se and Fe were greater for cows in MM compared with cows in POS. Cows in MM tended to have greater plasma glutathione peroxidase activity compared with cows in POS. An upregulation of liver *GPX1* was observed for cows in POS compared with cows in MM. In conclusion, subcutaneous injection of trace minerals maintained an adequate antioxidant response when an AF challenge was present.

Key words: aflatoxin, liver, trace minerals, AFM1

INTRODUCTION

An estimated $0.11 to $1.68 billion is lost annually due to the effects of mycotoxins on corn crops (Mitchell et al., 2016). Mycotoxins are toxins produced by fungi growing on feed crops such as corn, with the 3 most common being aflatoxin (**AF**), fumonisin, and deoxynivalenol (Flores-Flores et al., 2015; Mitchell et al., 2016). Aflatoxin B_1 (**AFB1**), an aflatoxin derivative produced by *Aspergillus parasiticus* and *flavus*, is hydroxylated and demethylated in the liver to aflatoxin M_1 (**AFM1**) after ingestion (Kuilman et al., 2000). Aflatoxin B_1 and AFM1 are classified as group 1 carcinogens by the International Agency for Research on Cancer (Liu and Wu, 2010); therefore, the FDA has set limits on AF concentration in feedstuffs and milk to be 20 and 0.5 μg/kg, respectively (Peraica et al., 1999).

Aflatoxin exposure causes adverse effects in dairy cattle, such as inappetence, immunosuppression, decreased milk production, and reproductive disorders (Abrar et al., 2013; Sulzberger et al., 2017). Aflatoxin B1 is believed to increase oxidative stress through the production of reactive oxidative species, primarily superoxide anions and hydrogen peroxides in the liver (Guengerich et al., 2001). Superoxide dismutase (**SOD**) is a Zn-, Mn-, and Cu-dependent enzyme linked to oxidative stress and the reduction of reactive oxidative species (Machado et al., 2014). Weatherly et al. (2018) observed greater plasma SOD concentrations for cows challenged with AF (2.77 U/mL) than cows not challenged (1.96 U/mL). Additionally, reports show that AFB1 increases bovine peripheral blood mononuclear

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cells gene expression of antioxidants, particularly SOD and glutathione peroxidase (**GSH-Px**), to combat the effects of oxidative stress (Bernabucci et al., 2011).

Trace minerals have an important role in immunological functions, antioxidant activity, and overall health in livestock (Spears and Weiss, 2008; Sordillo, 2013). Trace mineral status in animals varies depending on physiological status, dietary source, inflammation, and interactions among dietary constituents (Herdt and Hoff, 2011). Liver tissue often reflects trace mineral status of livestock, and low hepatic trace mineral concentration can lead to decreased inflammatory related enzyme activity (Kincaid, 2000; Herdt and Hoff, 2011). Stressful conditions, such as those experienced during the transition period, cause a decrease in DMI, which could subsequently affect trace minerals status (Drackley, 1999; Mulligan et al., 2006). Supplementing trace minerals via injection independently from DMI, has been proven to minimize stress-related issues by offering consistent trace mineral status (Vanegas et al., 2004; Machado et al., 2013). Injectable trace minerals (Zn, Mn, Se, and Cu) administered to dairy cows decreased the incidence of mastitis from 25 to 20% and endometritis from 34 to 29% compared with cows that did not receive the injectable trace minerals (Machado et al., 2013). Additionally, cows without trace mineral injection had lower SOD serum activity (12.7 U/mL) than cows administered injectable trace minerals (16.0 U/mL; Machado et al., 2014). Therefore, administration of injectable trace minerals allows for consistent trace mineral status while aiding the antioxidant response during stressful conditions (Teixeira et al., 2014).

Although it is known that trace minerals play a vital role in the immune system during oxidative stress, limited research exists regarding the relationship between trace mineral supplementation and AF exposure in dairy cows (Sordillo, 2013). Therefore, the objective of our study was to evaluate the effects of 2 subcutaneous injections of 15 mg/mL of Cu, 5 mg/mL of Se, 60 mg/mL of Zn, and 10 mg/mL of Mn (Multimin 90, Multimin North America, Fort Collins, CO) given at 1 mL/90.7 kg of average BW on lactating multiparous Holstein cow performance, blood chemistry, liver mineral concentration, and liver inflammatory markers during an aflatoxin challenge.

MATERIALS AND METHODS

Animal Care and Housing

All experimental procedures were approved by the University of Illinois (Urbana-Champaign) Institutional Animal Care and Use Committee (#16139). The experimental period occurred from November 2016 to March 2017. Cows were fed ad libitum for a 5% minimum refusal and had constant access to water. Diet (TMR) was formulated according to NRC (2001) recommendations (Table 1) based on cows at 180 DIM, 703 kg of BW, producing 32 kg of milk/d with a target of 3.8% milk fat and 3.3% milk protein, and a predicted DMI of 25 kg/d.

Table 1. Ingredient composition of the lactation diet fed to cows with negative control with no mineral injection (NEG), positive control with no mineral injection (POS), and treatment with mineral injection of Multimin 90 (MM) throughout the study

Ingredient	% of DM
Corn silage[1]	36.37
Canola meal	11.71
Alfalfa hay	11.20
Corn gluten feed	8.29
Soy hulls	4.29
Wheat straw	2.34
Dry ground corn grain	19.25
Blood meal	1.89
Rumen-protected lysine[2]	0.62
Rumen-protected methionine[3]	0.15
Potassium carbonate	0.13
Sodium bicarbonate	1.31
Calcium	1.08
Potassium chloride	0.44
Urea 46%	0.33
Salt, white	0.20
Magnesium oxide 54%	0.19
Vitamin and mineral mix[4]	0.22

[1]All treatments fed at 34.2% corn silage DM.

[2]Ajipro-L Generation 2 (Ajinomoto Heartland Inc., Chicago, IL).

[3]Smartamine M (Adisseo, Alpharetta, GA).

[4]Vitamin and mineral mix was formulated to contain 12.51% Ca, 14.06% Na, 9.60% Cl, 3.18% Mg, 6.48% K, 0.19% S, 26.93 mg/kg of Co, 301.01 mg/kg of Cu, 40.22 mg/kg of I, 678.25 mg/kg of Fe, 1,519.35 mg/kg of Mn, 8.62 mg/kg of Se, 4.47 mg/kg of organic Se, 1621.05 mg/kg of Zn, 43.34 kIU/kg of vitamin A, 10.89 kIU/kg of vitamin D_3, 466.41 IU/kg of vitamin E, 4.23 mg/kg of biotin, 46.65 mg/kg of thiamine, and 0.35 g/kg of monensin (Rumensin, Elanco, Greenfield, IN).

Experimental Design and Aflatoxin Challenge Procedure

A total of 58 multiparous Holstein cows [BW (mean ± SD) = 734 ± 60 kg; DIM = 191 ± 93] were assigned to 1 of 3 treatments in a randomized complete block design consisting of 19 blocks. Cows were distributed into blocks with regard to lactation number, DIM, previous lactation 305-d milk yield, and BCS. Experimental period (63 d) was divided in an adaptation phase (d 1–56) and a measurement phase (d 57–63). From d 57 to 59, cows received an AF challenge directly after feeding at 0700 h. The AF challenge was similar to the one proposed by Kutz et al. (2009). Dietary AF was obtained from the Veterinary Medical Diagnostic Laboratory, College Veterinary Medicine, University of