J. Dairy Sci. 99:1039–1046
http://dx.doi.org/10.3168/jds.2015-10242

Effects of calcium montmorillonite clay and aflatoxin exposure on dry matter intake, milk production, and milk composition

C. R. Maki,* A. D. Thomas,† S. E. Elmore,* A. A. Romoser,* R. B. Harvey,‡§ H. A. Ramirez-Ramirez,†§ and T. D. Phillips*§[1]

*College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station 77843
†Department of Animal Science and Veterinary Technology, Tarleton State University, Stephenville, TX 76402
‡Food and Feed Safety Research, Agricultural Research Service, USDA, College Station, TX 77845
§Texas A&M AgriLife Research, College Station 77843

ABSTRACT

Fifteen primiparous crossbred dairy cows that were 114 ± 14 d in milk and weighed 533 ± 56 kg were used in a replicated 5 × 5 Latin square to test the efficacy of a calcium montmorillonite clay, NovaSil Plus (NSP; BASF Corp., Ludwigshaven, Germany), for the reduction of aflatoxin (AF) metabolite (AFM_1) in milk and the effect of NSP on milk composition. Cows were housed in a freestall barn, fed once a day and milked twice a day. The experiment consisted of five 14-d periods: d 1 through 7 were considered for data collection, and d 8 through 14 were considered a wash-out phase. In each period, cows were randomly assigned to 1 of 5 dietary treatments: (1) control (CON), consisting of a basal total mixed ration (TMR); (2) high-dose NSP diet (NSP-1%), consisting of TMR plus 230 g of NSP; (3) aflatoxin diet (AFD), consisting of the TMR plus AF challenge; (4) low-dose NSP with AF (NSP-0.5%+AFD), composed of TMR plus 115 g of NSP and AF challenge; and (5) high-dose NSP with AF (NSP-1%+AFD), consisting of TMR plus 230 g of NSP and AF challenge. The AF challenge consisted of top dressing a daily dose of 100 μg/kg estimated dry matter intake (DMI); similarly, NSP was fed at 1.0 or 0.5% of estimated DMI. Milk yield and DMI were similar across treatments averaging 21.1 ± 1.33 kg/d and 19.7 ± 0.56 kg/d, respectively. Concentration of milk fat, protein, and lactose were similar across treatments with averages of 4.91 ± 0.20%, 3.85 ± 0.10%, and 4.70 ± 0.06%, respectively. Concentration of vitamin A averaged 0.28 ± 0.03 μg/mL and riboflavin concentration averaged 1.57 ± 0.13 μg/mL across treatments. The concentration of minerals in milk were similar for all treatments. Cows fed CON and NSP-1% yielded the lowest concentration of AFM_1 in milk with 0.03 and 0.01 ± 0.06 μg/L. Addition of NSP reduced milk AFM_1 from 1.10 ± 0.06 μg/L with the AF diet to 0.58 and 0.32 ± 0.06 μg/L with the NSP-0.5%+AF and NSP-1%+AF diets, respectively. Excretion of AFM_1 was reduced by NSP; mean values were 24.38, 11.86, 7.38, 0.64, and 0.23, ± 1.71 μg/d, for AFD, NSP-0.5%+AFD, NSP-1%+AFD, NSP-1%, and CON, respectively. More specifically, 1.07 ± 0.08% of the daily AF intake was transferred to the milk of cows consuming the AFD, whereas the AF transfer rates in milk from cows that consumed the NSP-0.5%+AFD and NSP-1%+AFD were 0.52 and 0.32 ± 0.08%. Results from this research demonstrate that feeding NSP to lactating cows is an effective method to reduce the transfer and excretion of AFM_1 in milk with no negative effects on dry matter intake, milk production, and composition.

Key words: aflatoxin, food safety, milk vitamins, mycotoxins

INTRODUCTION

Aflatoxins (**AF**) are harmful secondary metabolites produced primarily by the fungi *Aspergillus flavus* and *Aspergillus parasiticus* (Kurtzman et al., 1987). These compounds and their derivatives are known to be immunosuppressive and carcinogenic in different species (Linsell and Peers, 1977; Peers et al., 1987). Feed contamination with AF may originate from pre- and postharvest contamination of crops or feeds. Pre-harvest contamination becomes more prevalent in periods of drought stress and high temperatures during the growing season of crops (Cotty and Jaime-Garcia, 2007), whereas postharvesting contamination may arise from storage conditions that promote fungal growth (Cavallarin et al., 2011).

When AF-contaminated diets are fed to lactating animals, the toxin is primarily metabolized in the liver into a hydroxylated derivative called AFM_1 (Kuilman et al., 1998), transported to the mammary gland via blood, and transferred to milk. Detection of AFM_1 may occur in periods as short as 5 min postingestion in plasma (Gallo et al., 2008) and 1 h in milk (Bat-

Received August 11, 2015.
Accepted November 4, 2015.
[1]Corresponding author: tphillips@cvm.tamu.edu

tacone et al., 2012). Because of its carcinogenic and toxic properties, the US Food and Drug Administration (FDA, 2000) has set an action level of 0.5 μg/L for AFM_1 in milk. Recent drought conditions in the United States have led to greater risk for preharvest AF contamination of crops destined for animal feed (Cotty and Jaime-Garcia, 2007; Guo et al., 2008); therefore, it is necessary to evaluate practical and inexpensive strategies to mitigate AF content in animal feed and animal products. Examples of feed additives that have been reported to decrease the bioavailability of AF from contaminated dairy rations include yeast-derived products (Firmin et al., 2011), clays such as Na and Ca bentonite (Diaz et al., 2004), or combinations of yeast products with clay (Kissell et al., 2013; Xiong et al., 2015). Of these products, montmorillonite clays have yielded the best results, particularly a hydrated calcium montmorillonite available under the trade name NovaSil and NovaSil Plus (**NSP**; Phillips et al., 1988; Harvey et al., 1991; Phillips et al., 2008, 2002; Kutz et al., 2009).

Effective feed additives must reduce the bioavailability of AF with no effects on animal performance nor the nutritional content of animal products. Although several studies have reported milk composition from cows consuming AF-contaminated diets, to this date, we are not aware of any experiments where milk vitamins and minerals (nutrient and nonnutrient) were evaluated during AF challenge and supplementation with NSP. Therefore, the objective of this study was to evaluate the effect of AF consumption in concert with dietary supplementation with NSP to reduce AFM_1 concentrations in milk and to determine responses on milk composition.

MATERIALS AND METHODS

Animal Care, Housing, and Feeding

The experimental cows were cared for according to the guidelines stipulated by the Institutional Animal Care and Use Committee of Tarleton State University (Stephenville, TX). Cows were housed in a freestall barn equipped with individual feeding gates (Calan Broadbent Feeding System, American Calan, Northwood, NH). Daily care involved milking at 0900 and 1900 h, individual feeding at 0700 h for approximately 110% ad libitum consumption, and orts were collected, weighed, and recorded individually. The experiment consisted of five 14-d periods in which d 1 through 7 of each period were considered for data collection and d 8 through 14 were considered a wash-out period to avoid carry-over effects. Body weight and BCS (1 to 5 scale) were measured on d 6 and 7 of each period after milking; the scoring method used was similar to that of Wildman et al. (1982), but reported to the quarter point. Body condition score was independently measured by 2 evaluators, and scores were averaged.

Animals, Experimental Design, and Treatments

Fifteen crossbred (Holstein × Jersey × Norwegian Red) primiparous dairy cows averaging (±SD) 114 ± 14 DIM and 533 ± 56 kg of BW were used in replicated 5 × 5 Latin squares. The test product was a calcium montmorillonite clay available under the trade name NovaSil Plus (BASF Corporation, Ludwigshafen, Germany). It was predicted that cows would consume 23 kg of DM; therefore, NSP was fed at low and high doses equivalent to 0.5 and 1.0% of predicted DMI; similarly, an AF challenge was carried out by feeding a daily dose of 100 μg/kg estimated DMI via a top-dressed supplement. The AF supplement was produced from rice fermentation by *A. parasiticus* NRRL 2999 as described by Shotwell et al. (1966) and modified by West et al. (1973). Fermented rice was autoclaved and ground and the AF content was initially determined by spectrophotometric analysis (Nabney and Nesbitt, 1965; Wiseman et al., 1967). Rice powder containing 758 mg of AFB_1/kg of weight was obtained from the Food and Feed Safety Research Facility, USDA/ARS, College Station, TX. The concentration of AF was verified by the Office of The Texas State Chemist, Texas A&M University (College Station, TX). Of the total AF content in the rice powder, 79% was AFB_1, 16% was AFG_1, 4% was AFB_2, and 1% was AFG_2. In each 14-d period, cows within a square were randomly assigned to 1 of 5 dietary treatments: (1) control (**CON**), consisting of a basal TMR (Table 1); (2) high-dose clay diet (**NSP-1%**), consisting of basal TMR plus 230 g of NSP; (3) AF diet (**AFD**), consisting of the basal TMR plus AF challenge; (4) low-dose clay with AF (**NSP-0.5%+AFD**), composed of basal TMR plus 115 g of NSP and AF challenge; or (5) high-dose clay with AF (**NSP-1%+AFD**), consisting of basal TMR plus 230 g of NSP and AF challenge.

Sampling and Data Collection

Feed Sampling. Samples of the basal TMR were collected on d 6 and 7 of each period and subsequently pooled by period. The Penn State Forage Particle Separator was used to determine TMR particle size distribution as described by Kononoff et al. (2003). Feed samples were dried at 65°C in a forced-air oven to determine DM. After determination of DM, samples were ground (1-mm screen; Wiley Mill, Arthur H. Thomas Co., Philadelphia, PA) and stored at room temperature. A subsample of composite TMR was