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

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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., Ludwigshafen, Germany), for the reduction of aflatoxin (AF) metabolite (AFM₁) 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 $4.91 \pm 0.20\%$, $3.85 \pm 0.10\%$, and $4.70 \pm 0.06\%$, 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 AFM₁ in milk with 0.03 and 0.01 ± 0.06 µg/L. Addition of NSP reduced milk AFM₁ 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 AFM₁ 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, $1.07 \pm 0.08\%$ 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 $0.32 \pm 0.08\%$. Results from this research demonstrate that feeding NSP to lactating cows is an effective method to reduce the transfer and excretion of AFM₁ 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 post-harvest 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 AFM₁ (Kuilman et al., 1998), transported to the mammary gland via blood, and transferred to milk. Detection of AFM₁ 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.

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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 $\mu\text{g/L}$ for AFM₁ 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 AFM₁ 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 $\times$ Jersey $\times$ Norwegian Red) primiparous dairy cows averaging ($\pm$ 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 $\mu\text{g/kg}$ 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 AFB₁/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 AFB₁, 16% was AFG₁, 4% was AFB₂, and 1% was AFG₂. 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

Table 1. Ingredient and analyzed chemical composition of basal diet

Item	Value
Dietary ingredient (% of DM)	
Corn silage	32.0
Alfalfa hay	3.4
Coastal grass hay	3.3
Sudan grass baleage	2.6
Ground corn	22.5
Beet pulp pellets	10.8
Cottonseed	6.0
Concentrate premix ¹	19.4
Chemical (% of DM)	
CP	16.3
NDF	29.7
Starch	27.4
Ether extract	4.8
NFC ²	44.16
Ash	6.8
Particle size distribution ³ (% retained in each sieve)	
>19.0 mm	6.0
19.0–8.0 mm	32.4
8.0–4.0 mm	20.9
<4.0 mm	40.7

¹Contained canola meal, soybean meal, corn dried distillers grains, molasses, expeller-cooked soybean meal (SoyPlus, West Central Cooperative, Ralston, IA), calcium salts of fatty acids (EnergII, Virtus Nutrition, LLC, Corcoran, CA), yeast and yeast culture media extracts (Diamond V XP, Diamond V Mills Inc., Cedar Rapids, IA), isopropyl ester of 2-hydroxy-4-methylthiobutanoic acid (MetaSmart Dry, Adisseo, Alpharetta, GA), sodium sesquicarbonate, mineral and vitamin mix, urea, di-calcium phosphate, sodium chloride, magnesium oxide, calcium carbonate, and monensin.

²NFC calculated by difference of $100 - (\% \text{ NDF} + \% \text{ CP} + \% \text{ ether extract} + \% \text{ ash})$.

³Measured using the Penn State Particle Separator (Nasco, Fort Atkinson, WI).

analyzed for nutrient composition by an external laboratory (Cumberland Valley Analytical Services, Hagerstown, MD). Analyses included DM (method 930.15; AOAC International, 2000), N (method 990.03; Leco FP-528 Nitrogen Combustion Analyzer, Leco Corp., St. Joseph, MI), NDF (Van Soest et al., 1991), starch (Hall, 2009), ether extract using diethyl ether as the solvent (method 2003.05; AOAC International, 2006), ash (method 942.05; AOAC International, 2000), and phosphorus by inductively coupled plasma (method 985.01; AOAC International, 2000).

Milk Data Collection. Individual milk production was measured and recorded daily; measurements from the first 7 d of each period were used to evaluate milk production; additionally, milk samples were collected during the morning and night milking of d 6 and 7. One aliquot of approximately 30 mL from each milking was preserved using a pellet of 2-bromo-2-nitropropane-1,3 diol at room temperature. Milk samples were analyzed for fat, true protein, lactose, and SNF (AOAC International, 2000) by an external laboratory (Texas DHIA, Canyon, TX), using a B2000 Infrared Analyzer (Bentley Instruments, Chaska, MN). Milk urea nitrogen was

determined by the same laboratory using a modified Berthelot reaction concentration using a ChemSpec 150 Analyzer (Bentley Instruments). Yields of milk components were estimated according to milk weight and time of collection.

Additionally, a composite milk sample was obtained for d 6 and 7 by mixing proportional aliquots from each milking. The composite sample from d 6 was analyzed for AFM₁ content, and the composite sample from d 7 was analyzed for vitamin content. Aliquots from the first period d 6 were analyzed for mineral concentration. For AF determination, milk samples were processed according to official AOAC International methods. Briefly, samples were warmed to 37°C, centrifuged for 20 min at $2,000 \times g$, and defatted. The samples were passed through a coffee filter to remove any residual fat. Then, a 10-mL aliquot of the defatted fraction was passed through an immunoaffinity column (Afla WB, Vicam, Milford, MA) at a steady gravity-controlled flow rate (approximately 1 mL/min). The columns were washed twice with 10 mL of double distilled, de-ionized water (MilliQ 18.2 MΩcm), and eluted with 4 mL of acetonitrile. Samples were evaporated to dryness under constant nitrogen. Samples were re-suspended in 1 mL of 1:1 MeOH:H₂O solution and analyzed by LC-MS/MS (Waters H-class UPLC-MS/MS with ESI capability) in the positive mode for AFM₁ (molecular weight 328). The mobile phase consisted of an isocratic gradient of 30% H₂O, 70% acetonitrile, and 0.1% formic acid at a flow rate of 0.325 mL/min. The column temperature and injection volume were 40°C and 10 μL, respectively. The AF standards were purchased from Sigma Chemical Co. (St. Louis, MO). All solvents were obtained from Fisher Scientific (Pittsburgh, PA). The AF levels were quantified with the instrument software (Empower 2, Waters Corporation, Milford, MA). Aflatoxin excretion and AF transfer were calculated based on milk production on the day of collection according to the following equations:

$$\text{AF excretion} = \frac{\text{concentration of AF}}{\text{in milk} \times \text{milk yield}},$$

$$\text{AF transfer} = \left(\frac{\text{AF excretion}}{\text{AF intake}} \right) \times 100.$$

Determination of vitamin A was carried out using AOAC official methods (AOAC International, 2000). Briefly, an aliquot of 10 mL of milk was added to a 150-mL centrifuge tube. Then, 30 mL of antioxidant solution (1% pyrogallol) and 5 mL of saponification solution (10.5 M potassium hydroxide) were added to the test tubes. Tubes were then capped and swirled briefly

to mix. The tubes were then placed in a shaking H₂O bath at 70°C set to 60 oscillations/min for 25 min. After mixing, samples were allowed to cool to room temperature and transferred to a 125-mL separatory funnel; complete transfer was ensured by rinsing the centrifuge tubes with 30 mL of H₂O into the funnel. Subsequently, 30 mL of extraction solvent hexane-methylene chloride (3+1) were added into the funnel and gently mixed for 2 min. The aqueous layer was then removed; this last step was performed in triplicate. A 20-mL aliquot was evaporated to dryness under nitrogen and resuspended in 5 mL of mobile phase. The analysis was performed by HPLC (Waters Corporation). In this protocol, 100 µL of sample were injected in a mobile phase consisting of hexane-isopropyl alcohol (100 + 0.25, vol/vol) with a flow rate of 1.5 ± 0.2 mL/min at ambient temperature. Peaks were detected via UV absorbance at 325 nm with a sensitivity 0.1 AUFS. The column was a 4.6 mm id $\times$ 15 cm stainless steel reversed-phase C-18 column with 5-µm particle size.

Concentration of riboflavin was determined according to official methods (AOAC International, 2000) following the protocol described by Ndaw et al. (2000). Briefly, 50 mL of 0.1 M hydrochloric acid was added to 5 mL of milk in a 250-mL conical flask. The samples were autoclaved at 121°C for 30 min and then allowed to cool to room temperature. Subsequently the pH was adjusted to 4.5 with 2.5 M sodium acetate followed by addition of 100 mg of Takadiastase (Pfaltz & Bauer, Waterbury, CT). The solution was incubated for 18 h in an oven at 45°C. After incubation, the solution was diluted to 100 mL with 0.01 M HCl and filtered with a 0.2-µm filter. Analysis was performed by HPLC using an injection volume of 50 µL of the filtrate at a flow rate of 1 mL/min. The HPLC system was equipped with a 250 $\times$ 4.6 mm i.d. reversed phase column with 5-µm particle size. The samples were run in isocratic mode using methanol:buffer (30:70) as the mobile phase. The buffer was a 0.5 M sodium acetate solution. Riboflavin was measured directly with fluorescence detection using excitation and emission wavelengths set at 422 and 522 nm, respectively.

For nutrient and nonnutrient mineral analysis in milk, 10-mL aliquots from the first treatment period were analyzed by the Veterinary Integrated Biosciences Metals Analysis Laboratory, College of Veterinary Medicine, Texas A&M University (College Station) using atomic absorption and inductively coupled plasma mass spectroscopy (ICP-MS; Afriyie-Gyawu et al., 2008). The metals that were analyzed in milk include Ca, K, Mg, Na, P, S, Ag, Al, As, B, Ba, Be, Cd, Co, Cr, Cu, Fe, Hg, Li, Mn, Mo, Ni, Pb, Sb, Se, Sr, Ti, U, V, and Zn. Briefly, tubes were shaken so that the samples were well mixed. Aliquots were freeze-dried

in a Labconco Freezone 12L lyophilizer. Dry milk was powdered and analyzed for Ag by a direct analysis method employing sample combustion in a stream of oxygen, trapping of Hg on gold, heating the gold trap to release amalgamated Hg, and analyzing Hg(0) in the vapor phase by atomic absorption using a Nippon MA-3000 (Nippon Instruments, College Station, TX). All other metals were measured by an aliquot of the powdered sample after digestion with nitric acid, hydrogen peroxide, and hydrochloric acid and then brought to volume with deionized water. Digestate was analyzed for several elements by inductively coupled plasma optical emission spectroscopy using a Spectro CirOS instrument (Fitchburg, MA). Digestate was then diluted by a factor of 10 and analyzed for the remaining 29 elements by ICP-MS using a Perkin Elmer DRC II instrument (Wellesley, MA).

Statistical Analysis

Data were analyzed as replicated 5 $\times$ 5 Latin squares using the GLIMMIX procedure of SAS (version 9.3, SAS Institute Inc., Cary, NC). The fixed effects of the model included the square, period within the square, and treatment. Random effects included cow within the square. The linear model for these data is written as follows:

$$y_{ijkm} = \mu + \tau_m + \beta(\tau)_{im} + \rho(\tau)_{jm} + \alpha_k + \varepsilon_{ijkm},$$

where y_{ijkm} is the observation $ijkm$; μ represents the overall mean; τ_m represents the fixed effect square m ; $\beta(\tau)_{im}$ represents the random effect of cow i within square m ; $\rho(\tau)_{jm}$ represents the fixed effect of period j within square m ; and α_k represents the fixed effect of treatment. The error term ε_{ijkm} was assumed to be normally, independently, and identically distributed, with variance σ_e^2 . Statistical significance for all treatments effects was declared at $P \leq 0.05$; trends are discussed at $P \leq 0.15$. All mean results are presented as least squares means $\pm$ the largest standard error of the mean unless stated otherwise.

RESULTS AND DISCUSSION

Excretion of AFM₁ in bovine milk occurs when cows consume AF-contaminated feedstuffs, particularly grains such as corn and cottonseed. Therefore, prevention of enteric AF absorption is a field-practical and viable strategy to reduce the content of AFM₁ in milk. This study investigated the effects of NSP on milk yield and composition of dairy cows fed an AF-contaminated diet. We hypothesized that supplementation with NSP is safe and would reduce AFM₁ concentration in milk

with no effects on lactational performance or milk composition.

Prior to initiation of the study, it was predicted that cows would consume 23 kg of DM. Consequently, we prepared fixed amounts of AF supplement and NSP doses. The daily dose of AF was 100 µg of AFB₁/kg of DM, and NSP dosages were 115 and 230 g/d, equivalent to 0.5 and 1.0% of estimated DMI. Upon completion of the experiment, DMI was similar ($P = 0.34$; Table 2) across treatments averaging 19.7 ± 0.56 kg, which is 3.3 kg less than anticipated. Therefore, total AFB₁ concentration in the TMR was 117 µg/kg and NSP supplementation was at 0.58 and 1.17% of actual DMI; total intake of AFB₁ remained at 2,300 µg/d across treatments. Our observations on DMI are in accordance with similar studies with dairy cows where daily intake of AFB₁ ranged from 1,725 µg (Queiroz et al., 2012) to 2,500 µg (Kutz et al., 2009) with no effect on DMI. Milk yield was also similar ($P = 0.75$) across treatments with an average of 21.1 ± 1.33 kg/d; Kutz et al. (2009) used a similar sorbent and reported no deleterious effects on milk yield. We observed similar concentrations of fat ($P = 0.17$), protein ($P = 0.38$), and lactose ($P = 0.22$) across all treatments. Overall, milk composition averaged $4.91 \pm 0.20\%$ fat and $3.85 \pm 0.10\%$ protein. This is in accordance with other studies, in which feeding an AF-contaminated diet did not affect milk composition of dairy cows (Kutz et al., 2009; Queiroz et al., 2012), goats (Smith et al., 1994), and ewes (Firmin et al., 2011; Battaccone et al., 2012).

Furthermore, vitamin A and riboflavin concentration in milk were similar across treatments and averaged 0.28 ± 0.03 and 1.57 ± 0.13 µg/mL, respectively. The riboflavin content is within the range reported by Poulsen et al. (2015), 1.93 µg/mL for Danish Jersey and 1.40 µg/mL for Danish Holstein. Also, the concentrations of all minerals analyzed in milk samples were similar across all treatment groups (Table 3). Based on these findings, data from the first period were taken as representative of mineral concentration throughout the study. We are not aware of similar studies in which responses in milk vitamins or minerals were evaluated during AF challenge and supplementation with NSP; therefore, this is the first report to confirm no interference with nutritional vitamins and minerals in milk due to NSP inclusion in the diet.

Data of AFM₁ content in milk are presented in Table 4 and Figure 1; we observed a dose-dependent reduction ($P < 0.01$) in concentration of AFM₁ in milk with the inclusion of NSP. Feeding AFD resulted in 1.10 ± 0.06 µg/L; this value was reduced by 47.3% (0.58 ± 0.06 µg/L) with the inclusion of NSP at 0.58% of DMI and by 70.9% (0.32 ± 0.06 µg/L) when NSP was fed at 1.17% of DMI. Similarly, Kutz et al. (2009) reported a 48% reduction in milk AFM₁ when feeding NSP at 0.56% of DMI. Novasil Plus has been reported to effectively adsorb AFB₁ in laboratory conditions at pH 6.5 (Marroquín-Cardona et al., 2011). Interestingly, this pH is close to the mean rumen pH observed in dairy cows; therefore, the reduction ($P < 0.01$) in AF transfer

Table 2. Effect of dietary addition of NovaSil Plus¹ on the performance of dairy cows consuming an aflatoxin (AF)-challenge diet

Item	Dietary treatment ²					SEM ³	P-value ⁴
	CON	NSP-1%	AFD	NSP-0.5%+AFD	NSP-1%+AFD		
DMI (kg/d)	20.00	19.40	19.80	19.90	19.30	0.56	0.34
Milk yield (kg/d)	21.40	21.20	21.30	21.20	20.60	1.33	0.75
3.5% FCM ⁵	25.30	25.50	25.30	24.90	24.50	1.50	0.88
Fat (%)	5.01	5.04	4.80	4.76	4.92	0.20	0.17
Fat yield (kg/d)	1.01	1.02	1.00	0.97	0.97	0.06	0.74
Protein (%)	3.86	3.90	3.86	3.79	3.84	0.10	0.38
Protein yield (kg/d)	0.78	0.79	0.80	0.79	0.76	0.05	0.81
SNF (%)	9.72	9.60	9.68	9.56	9.66	0.13	0.44
Lactose (%)	4.77	4.61	4.72	4.67	4.73	0.08	0.22
Lactose yield (kg/d)	0.99	0.96	1.00	0.99	0.96	0.07	0.89
Vitamin A (µg/mL)	0.28	0.27	0.28	0.28	0.29	0.03	0.91
Riboflavin (µg/mL)	1.67	1.57	1.59	1.51	1.52	0.13	0.87
MUN (mg/dL)	11.66	12.02	11.24	10.74	11.20	1.13	0.29
BW (kg)	541.00	540.00	543.00	538.00	541.00	13.69	0.79
BCS ⁶	3.22	3.27	3.14	3.09	3.18	0.12	0.52

¹NovaSil Plus (BASF Corp., Ludwigshaven, Germany) is a calcium montmorillonite clay.

²CON = basal TMR; NSP-1% = basal TMR + 230 g of clay; AFD = basal TMR + 117 µg of AFB₁/kg of DMI; NSP-0.5%+AFD = basal TMR + 115 g of clay + 117 µg of AFB₁/kg per d of DMI; NSP-1%+AFD = basal TMR + 230 g of clay + 117 µg of AFB₁/kg of DMI.

³Highest standard error of treatment mean is shown.

⁴Main effect of treatment.

⁵3.5% FCM = [milk fat (kg) × 16.216] + [milk yield (kg) × 0.4324].

⁶1–5 scale (Wildman et al., 1982).

Table 3. Effect of dietary addition of NovaSil Plus¹ on nutrient and nonnutrient minerals in milk of dairy cows consuming an aflatoxin (AF)-challenge diet

Mineral (mg/kg per dw) ²	Dietary treatment ³					SEM ⁴	P-value ⁵
	CON	NSP-1%	AFD	NSP-0.5%+AFD	NSP-1%+AFD		
Ca	6,746.67	7,296.67	7,410.00	7,166.67	7,680.00	1,013.40	0.95
K	7,890.00	8,310.00	8,603.33	9,010.00	8,863.33	1,099.10	0.81
Mg	781.33	774.00	726.00	844.33	808.00	104.09	0.78
Na	2,360.00	2,166.67	2,463.33	2,830.00	2,603.33	479.80	0.67
P	5,693.33	5,893.33	6,450.00	6,050.00	7,073.33	697.07	0.35
S	2,176.67	2,236.67	2,296.67	2,213.33	2,410.00	153.01	0.36
Ag	<0.01	<0.01	<0.01	<0.01	<0.01	5.6e ⁻⁴	0.85
Al	<0.98	<1.01	<0.97	<0.97	<0.99	5.6e ⁻²	0.85
As	<0.10	<0.10	<0.10	<0.10	<0.10	5.6e ⁻³	0.85
B	0.70	0.95	0.90	0.93	0.82	0.22	0.72
Ba	0.47	0.63	0.56	0.48	0.60	0.13	0.30
Be	<0.05	<0.05	<0.05	<0.05	<0.05	2.7e ⁻³	0.85
Cd	<0.01	<0.01	<0.01	<0.01	<0.01	5.6e ⁻⁴	0.86
Co	<0.02	<0.02	<0.02	<0.02	<0.02	1.3e ⁻³	0.40
Cr	<0.05	<0.05	<0.05	<0.05	<0.05	2.7e ⁻³	0.85
Cu	0.41	0.40	0.64	0.43	0.45	7.9e ⁻²	0.14
Fe	2.77	3.12	2.78	3.13	3.90	0.67	0.22
Hg	<0.01	<0.01	<0.01	<0.01	<0.01	2.2e ⁻³	0.20
Li	<0.20	<0.20	<0.19	<0.19	<0.20	0.01	0.85
Mn	0.15	0.17	0.17	0.16	0.19	0.05	0.94
Mo	0.31	0.33	0.32	0.35	0.33	0.05	0.92
Ni	<0.10	<0.10	<0.10	<0.10	<0.10	5.6e ⁻³	0.85
Pb	<0.05	<0.05	<0.05	<0.05	<0.05	2.7e ⁻⁵	0.85
Sb	<0.01	<0.01	<0.01	<0.01	<0.01	3.8e ⁻³	0.72
Se	0.43	0.47	0.46	0.42	0.45	0.04	0.73
Sr	3.13	5.43	3.35	3.37	5.43	1.00	0.07
Ti	<0.01	<0.01	<0.02	<0.01	<0.01	6.7e ⁻³	0.50
U	<0.01	<0.01	<0.01	<0.01	<0.01	1.6e ⁻³	0.62
V	<0.05	<0.05	<0.05	<0.08	<0.05	2.7e ⁻³	0.85
Zn	30.57	35.57	35.87	27.40	32.93	3.77	0.13

¹NovaSil Plus (BASF Corp., Ludwigshaven, Germany) is a calcium montmorillonite clay.²Dry weight (dw) of freeze-dried milk powder.³CON = basal TMR; NSP-1% = basal TMR + 230 g of clay; AFD = basal TMR + 117 µg of AFB₁/kg of DMI; NSP-0.5%+AFD = basal TMR + 115 g of clay + 117 µg of AFB₁/kg per d of DMI; NSP-1%+AFD = basal TMR + 230 g of clay + 117 µg of AFB₁/kg of DMI.⁴Highest standard error of treatment mean is shown.⁵Main effect of treatment.

in this experiment may be explained by adsorption of AF in the rumen, thus rendering the toxin unavailable for absorption in the lower gastrointestinal tract. Specifically, transfer rate was reduced from 1.07% with the

AF diet to 0.52% and $0.32 \pm 0.08\%$ with the inclusion of NSP at 0.58% and 1.17% of DMI. Similar transfer rates have been reported for dairy cows consuming AF-contaminated diets (Harvey et al., 1991; Xiong et al.,

Table 4. Effect of dietary addition of NovaSil Plus¹ on aflatoxin (AF) M₁ content in milk from dairy cows consuming an AF-challenge diet

Item	Dietary treatment ²					SEM ³	P-value ⁴
	CON	NSP-1%	AFD	NSP-0.5%+AFD	NSP-1%+AFD		
AFM ₁ (µg/L)	0.03 ^d	0.01 ^d	1.10 ^a	0.58 ^b	0.32 ^c	0.06	<0.01
Excretion (µg/d)	0.64 ^c	0.23 ^c	24.38 ^a	11.86 ^b	7.38 ^b	1.71	<0.01
Transfer (%)	NA	NA	1.07 ^a	0.52 ^b	0.32 ^b	0.08	<0.01

^{a-d}Values in the same row with different superscripts differ.¹NovaSil Plus (BASF Corp., Ludwigshaven, Germany) is a calcium montmorillonite clay.²CON = basal TMR; NSP-1% = basal TMR + 230 g of clay; AFD = basal TMR + 117 µg of AFB₁/kg of DMI; NSP-0.5%+AFD = basal TMR + 115 g of clay + 117 µg of AFB₁/kg per d of DMI; NSP-1%+AFD = basal TMR + 230 g of clay + 117 µg of AFB₁/kg of DM.³Highest standard error of treatment mean is shown.⁴Main effect of treatment.

Figure 1. Boxplot representing the interquartile range and distribution of the data from 15 cows sampled at independent time frames in the Latin square design. CON = basal TMR; NSP-1% = basal TMR + 230 g of clay; AFD = basal TMR + 117 µg of aflatoxin (AF) B₁/kg of DMI; NSP-0.5%+AFD = basal TMR + 115 g of clay + 117 µg of AFB₁/kg per d of DMI; NSP-1%+AFD = basal TMR + 230 g of clay + 117 µg of AFB₁/kg of DMI. Boxes represent the interquartile range (IQR) between first and third quartiles and the line inside represents the median. The whiskers denote the lowest and highest values within $1.5 \times$ IQR from the first and third quartiles, respectively. Dots represent data points and outliers beyond the whiskers.

2015). Because of reduced transfer rate, total excretion of AFM₁ was also reduced ($P < 0.01$) in a dose-dependent manner. Cows that consumed AFD excreted 24.38 µg/d, whereas cows consuming the NSP-0.5%+AFD and NSP-1%+AFD excreted 11.86 µg/d and 7.38 ± 1.71 µg/d, which was equivalent to 51.3% and 69.7% reduction, respectively. It is important to note that the cows in this study were given diets contaminated with 117 µg/kg. This level is more than 5 times the US FDA regulatory standard of 20 µg/kg. Dairy feeds are expected to contain AFB₁ at levels less than 100 µg/kg. At these levels, assuming a 1% average carry-over of AF, the concentration of AFM₁ in milk should fall below the FDA action level of 0.5 µg/L. It is also noteworthy that background levels of AFM₁ were detected, even in milk from cows consuming the control diet. This finding confirms the presence of naturally occurring AF in the basal TMR and indicates a need for economically feasible and practical strategies to mitigate this toxin

in milk. In future studies, we intend to investigate the ability of NSP to reduce other AF biomarkers in milk.

CONCLUSIONS

Feed contamination with AF is of special concern in dairy animals due to the inherent risk of increased AFM₁ in dairy products intended for human consumption. Our data demonstrate that feeding NSP at 0.58 and 1.17% of DMI is a safe and effective strategy to reduce concentration and excretion of AFM₁ in milk, with no deleterious effects on milk production and composition including vitamin A, riboflavin, and mineral content.

ACKNOWLEDGMENTS

This work was supported by funding through the Aflatoxin Mitigation Center of Excellence Research Pro-

gram (National Corn Growers Association, St. Louis, MO), M1403049.

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