

Reduction of aflatoxin transfer into milk of lactating dairy cows with addition of a dietary clay adsorbent

S. C. Allen,¹ MS; B. J. Rude,² MS, PhD; R. H. Bailey,³ MS, PhD; S. H. Ward,¹ MS, PhD

¹ Department of Animal Science, North Carolina State University, Raleigh, NC 27695

² Department of Animal and Dairy Sciences, Mississippi State University, Mississippi State, MS 39762

³ College of Veterinary Medicine, Mississippi State University, Mississippi State, MS 39762

Corresponding author: Stephanie Hill Ward; Polk Hall 111D, Box 7261, Raleigh, NC 27695; 919-515-4015;

shward@ncsu.edu

Abstract

Twenty-four lactating Holstein cows were used in a randomized complete block design to test the efficacy of clay at reducing aflatoxin M₁ (AFM1) in milk. Cows were blocked by parity and stage of lactation and were adapted to individual feed gates prior to treatment. Cows were randomly assigned 1 of 4 dietary treatments (n=6): 1) control (CON) total mixed ration (TMR); 2) aflatoxin control (AFC) TMR with 300 ppb aflatoxin B₁ (AFB1); 3) adsorbent diet (CLY) TMR with 1.76 oz (50 g) of clay; 4) AFC diet with clay (CLY+AF) with 300ppb AFB1 and 1.76 oz (50 g) of clay. Data were analyzed using the GLM procedure of SAS. Main effects were treatment, days-in-milk, parity, and day. Significance was declared at P<0.05. Dry matter intake was similar across treatments; however, cows consuming CON diets had reduced milk yield. Milk from AFC cows had the greatest concentration of AFM1, and cows fed CLY+AF averaged 1.47 ± 0.186 ppb AFM1 less than cows fed AFC, resulting in a 60.0% reduction. Results from this study show that adding clay to contaminated diets was effective at reducing AFM1 concentrations in milk of cows fed AFB1 without negatively affecting production.

Key words: aflatoxin, bentonite, milk composition, mycotoxins, mitigation

Résumé

L'efficacité de l'argile à réduire l'aflatoxine M₁ (AFM1) dans le lait a été testée chez 24 vaches Holstein en lactation avec un plan expérimental avec blocs aléatoires complets. La parité et le stade de lactation ont servi de blocs et les vaches ont été accoutumées à des barrières individuelles d'alimentation avant le traitement. Les vaches ont été attribuées au hasard à l'un des quatre traitements d'alimentation suivants (n = 6) : 1) ration totale mélangée (TMR) témoin (CON), 2) TMR témoin d'aflatoxine (AFC) avec 300 ppm d'aflatoxine B₁ (AFB1), 3) TMR régime adsorbant (CLY) avec 1.76 onces (50 g) d'argile, et 4) régime AFC avec

argile (CLY+AF) avec 300 ppm d'AFB1 et 1.76 onces (50 g) d'argile. Les données ont été analysées avec la procédure GLM de SAS. Les effets fixes étaient le traitement, les jours en lait, la parité et le jour. Le seuil alpha était de 5%. La prise de matière sèche ne variait pas selon le traitement. Toutefois, la production de lait était moindre chez les vaches du groupe CON. La plus forte concentration d'AFM1 se retrouvait dans le lait des vaches AFC. La concentration moyenne d'AFM1 était plus basse par 1.47 ± 0.186 ppm chez les vaches du groupe CLY+AF par rapport aux vaches du groupe AFC, une réduction de 60%. Les résultats de cette étude montrent que l'ajout d'argile dans les diètes contaminées peut réduire efficacement la concentration d'AFM1 dans le lait de vaches nourries avec AFB1 sans impact négatif pour la production.

Introduction

Aflatoxins (AF) are a group of secondary metabolites predominately produced by species of *Aspergillus*, and are commonly found in corn, peanuts, and cottonseed.¹¹ Aflatoxin B₁ (AFB1), which is the most toxic of the 4 naturally occurring forms (aflatoxin B₁, B₂, G₁, and G₂), is considered a group 1 carcinogen.⁷ Once absorbed, AFB1 undergoes cytochrome P450-mediated oxidation, producing hydroxylated metabolites such as aflatoxin M₁ (AFM1), which can be transferred into the milk. When consumed by humans, AFM1 is considered potentially carcinogenic and is classified as a group 2B carcinogen.⁷ Processing of milk has variable results on AFM1 concentration,^{14,17} and AFM1 has been observed in numerous food products including infant formula, dried milk, cheese, yogurt, and in milk products from various animals, including human breast milk.⁶ Because of its carcinogenic properties, the FDA has established action limits of 0.5 and 20 ppb for AFM1 in milk and AFB1 in lactating cow feeds, respectively.⁵

Sequestering agents, specifically certain clays, can be used to detoxify AF-contaminated feed. Dietary additions of clay adsorbents have been reported to reduce the transfer of AFM1 into the milk of cows consuming diets containing AFB1 without negatively affecting production.^{8,9,12,18}

Although the ability of clay adsorbents to reduce AFM1 content in milk has been investigated in previous literature, efficacy varies depending on the type of clay and dose administered. The objectives of this study were to evaluate the efficacy of a bentonite clay with greater than 80% smectite content,³ at reducing the transfer of AF from contaminated feed into the milk of lactating dairy cows and its effects on production. The authors hypothesize that dietary addition of bentonite clay will reduce the transfer of AFM1 into the milk of lactating Holsteins without negatively affecting milk production.

Materials and Methods

Experimental Design and Management of Cows

This study was conducted at the Mississippi Agriculture and Forestry Experiment Station, Joe Bearden Dairy Research Center (Starkville, MS) under the approval of the Mississippi State University Animal Care and Use Committee (17-169-A) in February 2016. Cows were housed in a free-stall pen with sand bedding. They were individually fed at 0530 and 1730 h, allowing for ad libitum intake, and milked at 0400 and 1600 h in a double 8 parallel milking parlor. Cows were trained to use individual feeding gates^b prior to treatment.

A total of 24 mid- to late-lactation Holstein cows were used in a randomized complete block design. Cows were stratified by parity, stage of lactation, and previous milk production. Mid- and late-lactation cows averaged 125 and 375 days-in-milk (DIM), respectively. The experiment consisted of a 7-day treatment period in which the efficacy of bentonite clay was tested. Previous studies utilizing Latin Square designs have reported elevated AFM1 concentrations with exposure to AF-contaminated diets for periods of 3 to 7 days.^{4,9,10,11,12,15,18} Cows were estimated to consume 66 lb (30 kg)/d dry matter (DM), and 1.76 oz (50 g) clay (per company recommendation) was added to respective diets. Cows were randomly assigned to 1 of 4 dietary treatments (n=6): 1) control (CON), basal TMR with no AF or clay; 2) AF control (AFC), basal TMR plus 300 ppb AF; 3) clay (CLY), basal TMR plus 1.76 oz (50 g) clay; 4) clay with AFC diet (CLY+AF), basal TMR plus 1.76 oz (50 g) clay and 300 ppb AF. All treatment additions were top-dressed and mixed into approximately the top third of feed offered at 0530 only.

Feed Sample and Analysis

Aflatoxin standards were purchased from the University of Missouri (Columbia, MO). Aflatoxin B₁ was produced using rice fermentation by *A. parasiticus* NLR 2999 according to the methods described by Shotwell et al.¹⁶

Feed and orts were sampled on days 2 and 5 and composited by treatment. Feed samples were dried at 149°F (65°C) until dry to determine DM. Samples were then ground through a 2 mm screen in a Thomas Wiley Mill^c and stored at room temperature. All feed samples were subjected to proximate analysis and analyzed for total DM, ash, crude protein,

NDF, and ADF¹ (Table 1). Feed efficiency (FE) was calculated using the following equation:

$$FE = \text{lb milk/lb DMI}$$

Milk Sample and Analysis

Milk samples were taken at both AM and PM milkings throughout the treatment period for AFM₁ analysis. Samples were frozen immediately after collection. Later, samples were thawed, mixed, and 5 mL of milk (2.5 mL from AM and 2.5 mL from PM milking, within day) was combined with 10 mL of acetonitrile and vortexed for 1 min. QuEChERS extraction packets^d were added, and the samples were vortexed for 1 min. Samples were then centrifuged for 5 min at 1500 x g. The supernatant was collected and analyzed using HPLC. Aflatoxin secretion was calculated by multiplying the concentration of AFM1 by the milk yield based on milk production the day of collection. Aflatoxin transfer was calculated for AFC and CLY+AF diets by dividing AFM1 secretion by the amount of AFB1 added and multiplying by 100. Calculations are shown by the following equations:

AF secretion = concentration of AFM1 in milk × milk yield

$$AF \text{ transfer} = \left(\frac{\mu\text{g AFM1 secreted}}{\mu\text{g AFB1 added}} \right) \times 100$$

Table 1. Ingredient and analyzed chemical composition of basal diet fed to lactating Holsteins to evaluate the ability of bentonite clay to mitigate aflatoxin transfer.

| Item | Percent DM* |
|--|-------------|
| Dietary ingredient | |
| Alfalfa baleage | 6.0 |
| Bermudagrass baleage | 2.0 |
| Corn silage | 39.0 |
| Bermudagrass hay | 1.0 |
| Whole cottonseed | 4.0 |
| Energy Booster ^{®†} | 1.0 |
| Concentrate premix [‡] | 47.0 |
| Basal TMR composition[§] | |
| DM, % | 55.8 |
| Ash, % | 8.1 |
| CP, % | 17.4 |
| NDF, % | 37.8 |
| ADF, % | 18.7 |

* DM = dry matter

[†] Hubbard feeds, Mankato, MN

[‡] Contained fine ground corn grain, 47.5% solvent soybean meal, corn distillers grain, ground soybean hulls, meglac, sodium bicarbonate, calcium carbonate, 42% cottonseed meal, pro-team 70, salt, calcium phosphate, magnesium oxide, DCAD plus, potassium magnesium sulfate, urea, potassium chloride, WARE beef and dairy vitamin premix, selenium, Bio Fix Plus, Zinpro 4 Plex C, Ware vitamins ADE, vitamin E, rumensin, and wheat middlings. (16% Dairy Feed, Ware Milling, Houston, MS)

[§] TMR = total mixed ration, CP = crude protein, NDF = neutral detergent fiber, ADF = acid detergent fiber

Additionally, a second sample was collected on days 1 and 3 of the treatment period, during the AM milking only. Broad Spectrum Microtabs II™ tablets^e containing 8 mg Bro-nopol and 0.30 mg Natamycin were added to the additional samples after each milking for preservation of the sample. These samples from days 1 and 3 of the treatment period were analyzed for fat, protein, solids, and somatic cell counts¹ at Mid-South DHIA Laboratories (Missouri), and results were averaged. Bently FTS Combi^f was used to analyze SCC and components at Mid-South DHIA Laboratories. Somatic cell counts were analyzed using flow cytometry and components were analyzed using Fourier Transform Spectrometer (infrared spectroscopy). Milk component yields were calculated by multiplying the concentration of milk components by milk yield (MY). Somatic cell count was converted to somatic cell score (SCS) using the following equation:

$$SCS = \text{LOG}_2 \left(\frac{SCC}{100} \right) + 3$$

Statistical Analysis

Data were analyzed using the GLM procedure of SAS.^g Treatment, stage of lactation, and parity were considered independent variables, and milk yield, DMI, nutrient intakes, AFM1 variables, and milk composition were dependent variables. Day was used as a repeated variable for intake, MY, milk composition, and AFM1 variables. Means were separated using Fisher's Least Significant Difference, and significance was declared when $P \leq 0.05$. Tendencies were discussed when $0.05 < P \leq 0.10$. All interactions were tested and stepwise eliminated when not significant. All data were presented as mean \pm the largest standard error. Data from 1 cow consuming the CON diet were omitted as a result of reduced intake, while milk production remained similar to other study cows. This implied that she was able to consume feed from other feeding bins and was no longer a good representation of the CON diet and was thus removed from all data analysis. Milk and milk components yield data from another cow consuming the CON diet was omitted due to reduced milk yield. Removal of the respective cows was determined through performance

of an outlier test. Because of the brevity of the study (7 d), cows were not replaced.

Results

Feed composition was similar across treatments, and the diet composition for the basal TMR is displayed in Table 1. Feed given during the treatment period averaged 55.8, 8.1, 17.4, 37.8, and 18.7 % DM, ash, crude protein, NDF, and ADF, respectively. Intake of DM and nutrients was similar across treatment with the exception of CP and NDF intake (Table 2). DMI averaged 69.4 (31.5), 71.4 (32.4), 67.9 (30.8), and 71.1 (32.3) \pm 1.60 (0.72) lb (kg)/d for cows consuming CON, AFC, CLY, and CLY+AF diets, respectively ($P > 0.05$; Table 2). Intake of CP averaged 12.1 (5.5), 12.7 (5.5), 11.6 (5.3), and 12.5 (5.6) \pm 0.26 (0.12) lb (kg)/d for CON, AFC, CLY, and CLY+AF diets, respectively. CP intake was greatest in cows consuming AFC diets, least in cows consuming CLY diets, and cows consuming CON and CLY+AF diets were intermediate ($P = 0.02$; Table 2). NDF intakes followed the same trends.

Cows consuming CON diets produced less milk (71.9 [32.6] lb [kg]/d) than cows consuming other treatments (79.1 [35.9] lb [kg]/d, respectively, $P < 0.001$; Table 3). Feed efficiency was not different across treatments, and averaged 1.24 lb ECM/lb DMI ($P > 0.05$, Table 3). Milk from cows consuming CLY+AF had the greatest percent fat (4.9%), and cows consuming CLY diets had the least percent fat (3.94% \pm 0.207 [0.094], respectively, $P < 0.001$), and CLY+AF cows yielded more fat than other treatments (3.88 [1.76] vs 3.27 [1.44] \pm 0.174 [0.079] lb [kg]/d, respectively, $P < 0.001$). Milk from cows consuming CON and CLY+AF diets had less percent protein (3.02%) compared to AFC and CLY treatments (3.14% \pm 0.027; $P < 0.001$), and cows consuming CON diets yielded less protein than all other treatments (2.18 [0.99] vs 2.43 [1.10] \pm 0.062 [0.028] lb [kg]/d; $P = 0.004$). Cows consuming CLY+AF had the greatest percent lactose in milk (4.90%), and cows consuming AFC diets had the least percent lactose (4.64% \pm 0.034; $P < 0.001$). Cows consuming CLY+AF yielded greater lactose (3.92 [1.78] lb [kg]/d) than cows consuming CON and

Table 2. Effect of dietary addition of clay* on intake of dairy cows consuming a known concentration of aflatoxin (AF)

| Intake, lb [†] | Treatment [‡] | | | | SEM [§] | P value [¶] |
|-------------------------|------------------------|-------------------|-------------------|-------------------|------------------|----------------------|
| | CON | AFC | CLY | CLY+AF | | |
| Dry matter | 69.4 | 71.4 | 67.9 | 71.1 | 1.60 | 0.14 |
| Crude protein | 12.1 ^{ab} | 12.7 ^b | 11.6 ^a | 12.5 ^a | 0.26 | 0.02 |
| Organic matter | 63.5 | 65.6 | 62.1 | 64.9 | 1.47 | 0.12 |
| NDF | 25.9 ^{ab} | 27.5 ^b | 25.7 ^a | 27.5 ^a | 0.61 | 0.01 |
| ADF | 12.6 | 13.1 | 12.4 | 12.5 | 0.28 | 0.16 |

* Bentonite clay with greater than 80 % smectite content (Special Nutrients, Miami, FL)

[†] NDF = neutral detergent fiber; ADF = acid detergent fiber

[‡] CON = basal TMR; AFC = basal TMR + 300 ppb AF; CLY = basal TMR + 1.76 oz (50 g) clay; CLY+AF = AFC + 1.76 oz (50 g) clay

[§] Greatest standard error of treatment mean

[¶] Main effect of treatment

^{ab} Values with different superscripts are significantly different

Table 3. Effect of dietary addition of clay* on performance of dairy cows consuming a known concentration of aflatoxin (AF).

| Item [†] | Treatment [‡] | | | | SEM [§] | P value [¶] |
|-------------------------------------|------------------------|--------------------|--------------------|---------------------|------------------|----------------------|
| | CON | AFC | CLY | CLY+AF | | |
| Milk yield, lb | 71.9 ^b | 79.4 ^a | 77.6 ^a | 80.2 ^a | 1.85 | <0.01 |
| FE, lb ECM [#] milk/lb DMI | 1.21 | 1.22 | 1.21 | 1.32 | 0.05 | 0.26 |
| Fat, lb | 3.24 ^b | 3.24 ^b | 3.04 ^b | 3.88 ^a | 0.174 | <0.001 |
| Fat, % | 4.48 ^{ab} | 4.01 ^{bc} | 3.94 ^c | 4.91 ^a | 0.207 | <0.001 |
| Protein, lb | 2.18 ^b | 2.49 ^a | 2.40 ^a | 2.40 ^a | 0.062 | 0.004 |
| Protein, % | 3.04 ^b | 3.16 ^a | 3.11 ^a | 3.00 ^b | 0.027 | <0.001 |
| Lactose, lb | 3.44 ^b | 3.70 ^{ab} | 3.64 ^b | 3.92 ^a | 0.104 | 0.012 |
| Lactose, % | 4.76 ^b | 4.64 ^c | 4.72 ^b | 4.90 ^a | 0.034 | <0.001 |
| Solids, lb | 6.28 ^b | 6.90 ^a | 6.75 ^a | 7.08 ^a | 0.181 | 0.017 |
| Solids, % | 8.72 | 8.69 | 8.74 | 8.87 | 0.054 | <0.051 |
| MUN, mg/dL | 9.98 ^b | 11.07 ^a | 9.93 ^b | 10.43 ^{ab} | 0.268 | <0.001 |
| SCS | 2.91 ^a | 1.84 ^b | 2.51 ^{ab} | 2.21 ^{ab} | 0.254 | 0.025 |

* Bentonite clay with greater than 80 % smectite content (Special Nutrients, Miami, FL).

[†] FE = feed efficiency (lb dry matter intake/lb milk); MUN = milk urea nitrogen; SCS = somatic cell score (LOG 2 (somatic cell count x 10³/100) + 3)

[‡] CON = basal TMR; AFC = basal TMR + 300 ppb AF; CLY = basal TMR + 1.76 oz (50 g) clay; CLY+AF = AFC + 1.76 oz (50 g) clay

[§] Greatest standard error of treatment mean

[¶] Main effect of treatment

[#] Energy Corrected Milk calculated as: 0.327*Milk Yield (lb)+12.95*Milk fat (lb)+7.2*Milk protein (lb)

^{abc} Values with different superscripts are significantly different

CLY diets (3.44 [1.56] and 3.64 [1.65] lb [kg]/d, respectively), and AFC cows were intermediate (3.70 [1.68] ± 0.104 [0.047] lb [kg]/d; *P*=0.012). Cows consuming CLY+AF tended to have a greater milk solids percent compared to cows consuming AFC (8.87 vs 8.69 ± 0.268%; *P*=0.051), and cows consuming CON diets yielded less solids than all other treatments (6.28 vs 6.91 ± 0.181 [2.85 vs 3.13 ± 0.082] lb [kg]/d, respectively; *P*=0.017). Milk urea nitrogen was greatest in cows consuming AFC diets, and least in cows consuming CON and CLY diets (*P*<0.001). Milk from cows consuming AFC diets had the smallest SCS, and CON diets had the greatest SCS (*P*=0.025). All other diets were intermediate. Somatic cell score averaged 2.91, 1.84, 2.51, and 2.21 ± 0.254 for CON, AFC, CLY, and CLY+AF diets, respectively.

Aflatoxin M₁ concentration averaged 0.18, 2.32, 0.08, and 0.85 ± 0.186 ppb for CON, AFC, CLY, and CLY+AF cows, respectively (*P*<0.001; Table 4). Daily averages of AFM₁ by

treatment are displayed in Figure 1. The greatest concentration of AFM₁ was observed in milk from cows consuming AFC diets, followed by cows consuming CLY+AF diets, and cows consuming CON and CLY had the least concentration of AFM₁ in milk (Table 4; *P*<0.05). Inclusion of bentonite clay in the diet resulted in a 63.4% reduction in AFM₁. Secretion of AFM₁ followed the same trend as AFM₁ concentration and averaged 5.45, 80.97, 2.75, and 32.00 ± 16.87 µg/d for CON, AFC, CLY, and CLY+AF, respectively (*P*<0.05). Adding bentonite clay to the diet contaminated with AF reduced the transfer of AFM₁ to milk from 1.15 to 0.46 ± 0.094% (*P*<0.05), and resulted in a 60.0% reduction in transfer.

Aflatoxin M₁ concentration varied by day and treatment (*P*<0.05; Figure 1). Day 1 was similar among treatments. On day 2, milk from cows consuming AFC diets contained the greatest concentration of AFM₁, followed by animals consuming CLY+AF diets. Milk from cows consuming CON and CLY

Table 4. Effect of dietary addition of clay* on performance of dairy cows consuming a known concentration of aflatoxin (AF).

| Item [†] | Treatment [‡] | | | | SEM [§] | P value [¶] |
|------------------------|------------------------|--------------------|-------------------|--------------------|------------------|----------------------|
| | CON | AFC | CLY | CLY+AF | | |
| AFM ₁ , ppb | 0.18 ^c | 2.32 ^a | 0.08 ^c | 0.85 ^b | 0.186 | <0.001 |
| Secretion, µg | 5.45 ^c | 80.97 ^a | 2.75 ^c | 32.00 ^b | 6.869 | <0.001 |
| Transfer, % | N/A | 1.15 | N/A | 0.46 | 0.094 | <0.001 |

* Bentonite clay with greater than 80% smectite content (Special Nutrients, Miami, FL).

[†] AFM₁ = Aflatoxin M₁; Secretion = AFM₁ concentration in milk x milk yield; Transfer = (µg AFM₁ secreted/µg AFB₁ added to AF and CLY+AF diets) * 100

[‡] CON = basal TMR; AFC = basal TMR + 300 ppb AF; CLY = basal TMR + 1.76 oz (50 g) clay; CLY+AF = AFC + 1.76 oz (50 g) clay

[§] Greatest standard error of treatment mean

[¶] Main effect of treatment

^{abc} Values with different superscripts are significantly different

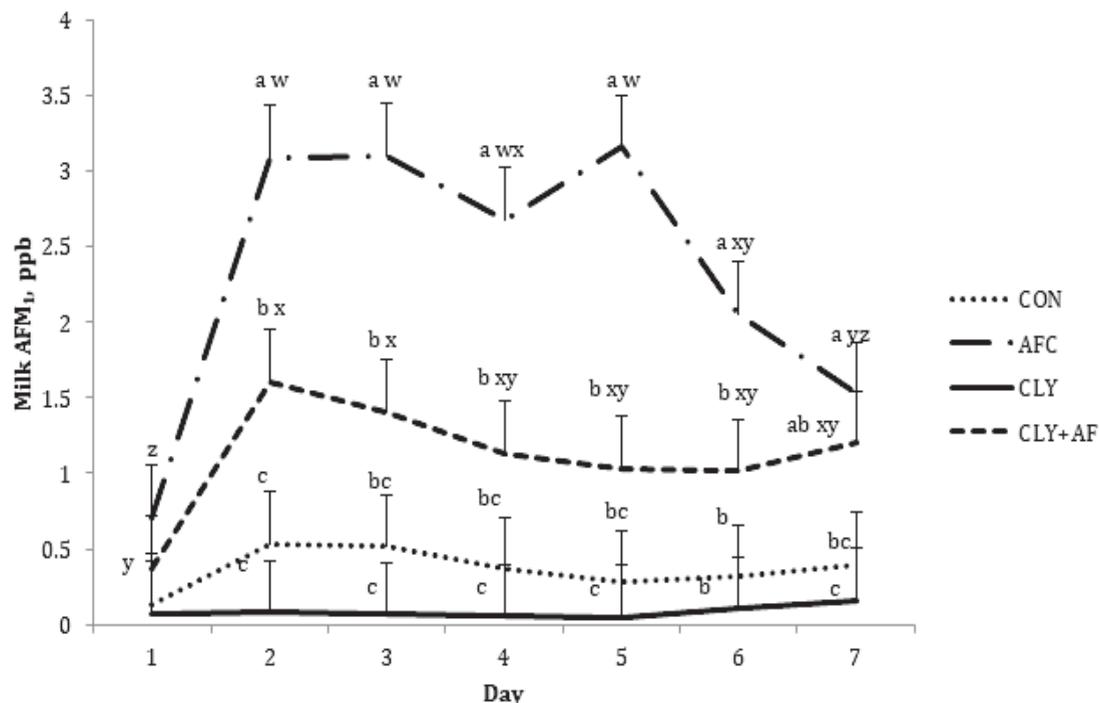


Figure 1. Daily aflatoxin (AF) M₁ concentrations throughout the treatment period in cows administered either 0 or 300 ppb AF and 0 or 1.76 oz (50 g) of a bentonite clay with greater than 80% smectite content (Special Nutrients, Miami, FL). CON = basal TMR; AFC = basal TMR + 300 ppb AF; CLY = basal TMR + 1.76 oz (50 g) clay; CLY+AF = AFC + 1.76 oz (50 g) clay. a-c represents differences between treatments within the same day. w-z represents differences between days within the same treatment.

diets were least concentrated in AFM₁. On days 3 through 5, and day 7, milk from cows consuming AFC diets contained the greatest concentration of AFM₁, followed by cows consuming CLY+AF. Milk from cows consuming CLY diets were least concentrated in AFM₁, and cows consuming CON diets were intermediate of CLY and CLY+AF cows. On day 6, cows consuming AFC diets contained the greatest concentration of AFM₁, and all other diets were similar. Aflatoxin M₁ concentration was similar during the treatment period for CON and CLY diets. Milk AFM₁ concentration was similar in cows consuming CON and CLY diets throughout the experiment. Cows consuming CLY+AF diets had the greatest concentration of AFM₁ in the milk on d 2 and 3, the least concentration of AFM₁ in the milk on day 1, and all other days were intermediate. Cows consuming AFC diets had the greatest concentration of milk AFM₁ on days 2, 3, and 5, and had the least concentration of milk AFM₁ on day 1.

Discussion

Elevated AFB₁ in AFC and CLY+AF diets confirmed the addition of dietary treatments. The reduction of AFB₁ in CLY+AF feeds may indicate that adsorption of AFB₁ by the bentonite clay occurred prior to preservation and analysis of samples. Effective sequestering agents must rapidly adsorb AF in order to reduce their bioavailability prior to absorption. However, accurate sampling for AF analysis is difficult

due to its heterogeneous nature of distribution in feed.¹⁸ Additionally, it is important to note that AFB₁ was detected in diets that did not receive AF treatments (CON and CLY), though great effort was made to prevent contamination across treatments and samples. Although both diets were below the action limit of 20 ppb, this emphasizes the occurrence of AF in dairy feeds and the importance of evaluating mitigation techniques.

Dry matter intake was not different between treatments; however, cows fed CLY diets did consume less CP and NDF compared to cows on other treatments. It is unlikely that feeding CLY would lead to a reduced CP or NDF intake and given that these cows also had greater MUN, we attributed this difference to sorting of feed and/or increased frequency of greater sorts, even though DMI was not different.

Two cows were removed from the CON treatment after outlier testing revealed their mean DMI and MY to be more than 2 SD's away from the mean. One cow was observed stealing feed from other Calan bins, indicating her data was not reflective of the CON treatment. The other cow was dropped due to reduced MY. We believe this was potentially due to behavior issues related to use of Calan bins. Milk yields for this cow returned to normal within 48 h of returning to the large pen. Although cows did have an adaptation period after training to Calan bins, before the data collection started, it is not unusual to see reduced performance in cows with any sort of restricted access to feed.

Cows consuming CON diets produced less milk than other treatments; we attribute this to the few number of cows fed the CON diet after removal of outliers. There was no consistent impact of clay or AFB₁ on milk components, with the exception of MUN, which was greatest in diets containing AFB₁ treatments. Given that diet composition was similar across treatments, with the exception of addition of AF and/or clay, and the brevity of this study, no change in milk components was expected. More frequent testing of components, as part of a longer study, could demonstrate a better relationship between AF consumption and milk components.

Cows consuming AFB₁ at the concentration used in the current study did not demonstrate a reduction in performance that would have been indicative of aflatoxicosis. This is consistent with previous studies.^{9,10,11} However, other studies have reported a reduction in MY,² milk fat yield¹⁵ and milk protein percent¹⁵ by cows consuming AF-contaminated diets with no clay compared to those not consuming AF. Battacone et al³ also reported a reduction in MY in ewes fed increasing concentrations of AFB₁ (0 and 32 vs 64 and 128 ppb).

The consumption of clay did not appear to negatively affect production. Numerous studies have reported that consumption of clay adsorbents did not alter DMI, MY, or milk composition when incorporated between 0.5 and 2.0% DMI.^{9,10,11,18} However, other studies have reported that inclusion of clay adsorbents may alter milk composition when incorporated between 0.2 and 1.0% DMI.^{12,15} Queiroz et al¹⁵ reported a reduction in milk protein percent as the concentration of clay increased from 0.2 to 1.0%; however, both diets containing clay and AFB₁ were similar to the control, which contained neither clay nor AFB₁. In contrast, Maki et al¹² reported that milk from cows consuming control diets containing no AFB₁ or clay had reduced percent fat than other diets containing clay. The authors also reported protein percent differed among diets, and was greatest in cows consuming AF-contaminated diets that were supplemented with 0.25% DMI of a clay adsorbent. Sulzberger et al¹⁸ reported a reduction in feed efficiency in cows administered a clay adsorbent; however, that was not observed in the current study.

Addition of bentonite clay effectively reduced the concentration, transfer, and secretion of AFM₁ compared to cows consuming AF diets with no clay. This reduction in AFM₁ following the dietary addition of a clay adsorbent is represented in numerous studies.^{4,9,10,11,12,18} Diaz et al⁴ concluded that sodium bentonites showed potential to effectively bind to AFB₁, and reduced transfer of AF ranging from 50 to 65%. Queiroz et al¹⁵ reported a reduction in AFM₁ concentration when clay was administered at 1.0% DMI. There was no difference between AFM₁ concentrations in the milk when cows were fed clay at 0.2% DMI compared to cows consuming AFB₁ and no clay. The authors also observed no differences between AF-contaminated diets containing no clay adsorbent and those in which a clay adsorbent was administered. However, a reduction was observed when the concentration of clay in the diet was increased to 1.0% compared to 0.2%

DMI. This contrasts with the current results; however, the previous authors administered a calcium montmorillonite clay and the current study evaluated a bentonite clay. Additionally, AFB₁ was administered at 75 ppb in the previous study, and the current study administered 300 ppb. Aflatoxin M₁ was not reduced below the action limit of 0.5 ppb; however, AFB₁ was administered at 15 times the action limit. Although AFM₁ concentrations were not reduced below the action limit, if the feed contamination were reduced further, the milk concentration may potentially fall below that limit. Additionally, dietary inclusion of bentonite clay averaged 0.142 and 0.136% for CLY and CLY+AF diets, respectively (1.76 oz [50 g] clay/average DMI). The inclusion of bentonite clay in this study is less than inclusion of clay observed in previous studies,^{9,10,11} yet the transfer of AFM₁ was reduced by 60.0% when clay was included in the diet. A similar dose of clay was used by Queiroz et al,¹⁵ and authors reported no reduction in AFM₁ concentration at this dose. Maki et al¹² reported that NovaSil Plus was successful at reducing AFM₁ in the milk of cows administered 50 ppb AFB₁ when fed at 0.125 and 0.25% DMI. The authors also reported that both doses were similar in reduction of AFM₁. However, previous studies using NovaSil Plus showed an increase in AFM₁ reduction as NovaSil Plus increased from 0.5 and 0.6 to 1.0 and 1.2% DMI.^{10,11} It would be interesting to evaluate the ability of bentonite clay to reduce AFM₁ if administered in doses greater than those used in the current study.

Although AFM₁ concentration was similar across treatments on d 1, this is expected as the first milk sample occurred prior to consumption of the treatments. An elevation of AFM₁ was expected to occur on d 2. It is interesting that a decrease in AFM₁ concentration in milk of AFC cows was observed on d 6 and 7, and on d 7 was similar to CON and CLY+AF diets. This response was unexpected, particularly since DMI was similar for AFC cows throughout the experiment. It is possible, however, that some cows fed the AFC diets were able to sort feed and avoid total consumption of the aflatoxin, given that it was added to the ration by hand.

Conclusions

The results of this study demonstrated the efficacy of bentonite clay at reducing AFM₁ transfer in the milk of cows consuming diets containing AFB₁. This study can also be used to further evaluate the ability and feasibility of including clay adsorbents in the diet of lactating cows when AF contamination occurs. Bentonite clay was included at a minimal concentration, and resulted in a 60.0% reduction of AFM₁ transfer when added to diets contaminated with 300 ppb AFB₁. This reduction occurred without altering intake, MY, or milk composition, indicating that this bentonite clay can be included at this concentration without compromising production. Although AFM₁ concentrations were not reduced below the action limit of 0.5 ppb, if the feed contamination were less severe, the milk concentration would most likely

fall below that limit. However, additional research is needed to determine the efficacy of bentonite clay at these concentrations. The importance of evaluating AF mitigation techniques was demonstrated by the presence of AFM1 in the milk from cows fed the control diet, indicating naturally occurring AF in the TMR on the farm. Further research is needed to evaluate bentonite clay at concentrations more closely resembling those that would be observed on dairy farms. Additionally, evaluating bentonite clay at different inclusion doses would determine if the dose fed during the current study was most effective, or if a greater dose would further reduce AFM1 transfer. Further research on inclusion of these adsorbents may benefit farmers that encounter AF contamination.

Endnotes

- ^a Mycoad, Special Nutrients, Miami, FL
^b Calan Broadbent Feeding System, American Calan, Northwood, NH
^c Thomas Wiley mill, model 4, Thomas Scientific, Swedesboro, NJ
^d Broad Spectrum Microtabs II™ tablets, Weber Scientific®, Hamilton, NJ
^e Bently FTS Combi, Chaska, MN
^f QuEChERS extraction packets, Phenomenex Inc., Torrance, CA
^g SAS® version 9.4, SAS Institute Inc., Cary, NC

Acknowledgements

The authors would like to thank Special Nutrients (Miami, FL) for funding this research and the Mississippi Agriculture and Forestry Experiment Station for providing the animals and facilities used in this research.

References

1. AOAC International. Official methods of analysis. 17th ed. Arlington, VA: AOAC Int, 2009.
2. Applebaum RS, Brackett RE, Wiseman DW, Marth EH. Responses of dairy cows to dietary aflatoxin: Feed intake and yield, toxin content, and quality of milk of cows treated with pure and impure aflatoxin. *J Dairy Sci* 1982; 65:1503-1508.
3. Battacone G, Nudda A, Cannas A, Cappio Borlino A, Bomboi G, Pulina G. Excretion of aflatoxin M1 in milk of dairy ewes treated with different doses of aflatoxin B1. *J Dairy Sci* 2003; 86:2667-2675.
4. Diaz DE, Hagler Jr WM, Blackwelder JT, Eve JA, Hopkins BA, Anderson KL, Jones FT, Whitlow LW. Aflatoxin binders II: Reduction of aflatoxin M1 in milk by sequestering agents of cows consuming aflatoxin in feed. *Mycopathologia* 2004; 157:233-241.

5. Food and Drug Administration. *Guidance for industry: Action levels for poisonous or deleterious substances in human food and animal feed, 2000*. Available at: www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/ChemicalContaminantsMetalsNaturalToxinsPesticides/ucm077969.htm#afla. Accessed Jan 30, 2016.
6. Galvano F, Galafaro V, Galvano G. Occurrence and stability of aflatoxin M₁ in milk and milk products: A worldwide review. *J Food Prot* 1996; 59:1079-1090. <http://dx.doi.org/10.4315/0362-028X-59.10.1079>.
7. International Agency for Research on Cancer. IARC Monographs on the evaluation of carcinogenic risks to human. Vol. 56. Some naturally occurring substances: Food items and constituents, heterocyclic aromatic amines and mycotoxins. International Agency for Research on Cancer, Lione, 1993.
8. Jiang Y, Ogunade IM, Kim DH, Li X, Pech-Cervantes AA, Arriola KG, Oliveira AS, Driver JP, Ferraretto LF, Staples CR, Vyas D, Adesogan AT. Effect of adding clay with or without a *Saccharomyces cerevisiae* fermentation product on the health and performance of lactating dairy cows challenged with dietary aflatoxin B₁. *J Dairy Sci* 2018; 101:3008-3020.
9. Kutz RE, Sampson JD, Pompeu LB, Ledoux DR, Spain JN, Vázquez-Añón M, Rottinghaus GE. Efficacy of Solis, NovasilPlus, and MTB-100 to reduce aflatoxin M₁ levels in milk of early to mid-lactation dairy cows fed aflatoxin B₁. *J Dairy Sci* 2009; 92:3959-3963. <http://dx.doi.org/10.3168/jds.2009-2031>.
10. Maki CR, Thomas AD, Elmore SE, Romoser AA, Harvey RB, Ramirez-Ramirez HA, Phillips TD. Effects of calcium montmorillonite clay and aflatoxin exposure on dry matter intake, milk production, and milk composition. *J Dairy Sci* 2016; 99:1039-1046. <http://dx.doi.org/10.3168/jds.2015-10242>.
11. Maki CR, Monteiro APA, Elmore SE, Tao S, Bernard JK, Harvey RB, Romoser AA, Phillips TD. Calcium montmorillonite clay in dairy feed reduces aflatoxin concentrations in milk without interfering with milk quality composition or yield. *Anim Feed Sci and Tech* 2016; 214:130-135. <http://dx.doi.org/10.1016/j.anifeeds.2016.02.014>.
12. Maki CR, Haney S, Wang M, Ward SH, Rude BJ, Bailey RH, Phillips TD. Calcium montmorillonite clay for the reduction of aflatoxin residues in milk and dairy products. *J Dairy and Vet Sci* 2017; 2:555587.
13. Pier AC. Major biological consequences of aflatoxicosis in animal production. *J Anim Sci* 1992; 70:3964-3967.
14. Purchase IFH, Steyn M, Rinsma R, Tustin RC. Reduction of the aflatoxin M content of milk by processing. *Fd Cosmet Toxicol* 1972; 10:383-387.
15. Queiroz OC, Han JH, Staples CR, Adesogan AT. Effect of adding a mycotoxins-sequestering agent on milk aflatoxin M₁ concentration and the performance and immune response of dairy cattle fed an aflatoxin B₁-contaminated diet. *J Dairy Sci* 2012; 95:5901-5908. <http://dx.doi.org/10.3168/jds.2011-5287>.
16. Shotwell OL, Hesseltine CW, Stubblefield RD, Sorenson WG. Production of aflatoxin on rice. *Appl Microbiol* 1996; 14:425-428.
17. Stoloff L, Trucksess M, Hardin N, Francis OJ, Hayes JR, Polan CE, Campbell TC. Stability of aflatoxin M in milk. *J Dairy Sci* 1975; 58:1789-1793.
18. Sulzberger SA, Melnichenko S, Cardoso FC. Effects of clay after an aflatoxin challenge on aflatoxin clearance, milk production, and metabolism of Holstein cows. *J Dairy Sci* 2017; 100:1856-1869. <https://doi.org/10.3168/jds.2016-11612>.
19. Wagner C. Critical practicalities in sampling for mycotoxins in feed. *J AOAC Int* 2015; 98:301-308.



FILLING THE GAPS IN DISEASE PREVENTION

Commercial vaccines are a vital part of any beef health protocol, but sometimes disease prevention requires a different approach. Newport Laboratories, Inc., creates custom-made vaccines designed to help fight the specific pathogens challenging your herd, ensuring your veterinary toolbox is always complete.

Learn more about custom-made vaccines at NewportLabs.com.



A Boehringer Ingelheim Company

The Newport Laboratories Logo® is a registered trademark of Newport Laboratories, Inc. ©2019 Newport Laboratories, Inc., Worthington, MN. All rights reserved. BOV-2035-NPL0419