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Effect of adding a mycotoxin-sequestering agent on milk aflatoxin M_1 concentration and the performance and immune response of dairy cattle fed an aflatoxin B_1-contaminated diet

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ABSTRACT

This project aimed to examine the effects of adding 2 doses of a montmorillonite-based mycotoxin adsorbent on milk aflatoxin M_1 (AFM_1) concentrations and the performance and innate immune response of dairy cows fed an aflatoxin B_1 (AFB_1)-contaminated diet. Eight lactating cows were used in a duplicated 4 × 4 Latin square design with 12-d periods. Treatments included the following: (1) control diet (C), (2) aflatoxin diet (T) containing C and 75 μg of AFB_1/kg, 3) low-clay (LC) diet containing T and Calibrin A (Amlan International, Chicago, IL) added at 0.2% of the diet dry matter (DM), and 4) high-clay diet (HC) containing T and Calibrin A added at 1% of the diet DM. Milk production and DM intake were recorded daily and milk was sampled twice daily on d 5, 9, 10, 11, and 12 in each period. Blood samples were collected on d 5 and 9 of each period. Dietary treatments did not affect DM intake, milk yield, or feed efficiency. Even though cows were limit fed, feeding T instead of C reduced milk fat yield (0.67 vs. 0.74 kg/d) and milk protein concentration (3.28 vs. 3.36%). Concentrations of AFM_1 in milk of cows fed the T and LC diets were similar (0.57 and 0.64 μg/kg) and greater than those of cows fed the HC diet (0.46 μg/kg). Haptoglobin concentration was greater (22.0 vs. 14.4) and β_2-integrin expression (220 vs. 131) tended to be greater in cows fed diet T instead of C, but values for cows fed LC, HC, and C did not differ. In comparison to C, feeding T increased the innate immune response and decreased milk fat yield and milk protein concentration, but feeding LC and HC did not affect these measures. Only the HC diet reduced milk AFM_1 concentration.

Key words: mycotoxin clay adsorbent, aflatoxin, immune response, milk

INTRODUCTION

Aflatoxins are secondary metabolites produced by *Aspergillus parasiticus*, *Aspergillus flavus*, and the rare *Aspergillus nomius* (Creppy, 2002). They occur in feeds as aflatoxin B_1 (**AFB_1**), B_2, G_1, and G_2 forms and in milk as their oxidative forms: M_1 and M_2 (Allcroft and Carnaghan, 1963; Hartley et al., 1963). Aflatoxins can negatively affect animal health, performance, and reproduction if consumed in sufficient quantities (Whitlow and Hagler, 2005b). Symptoms of chronic aflatoxin intoxication in cattle include decreased appetite, weight loss, milk yield, and feed efficiency and liver damage (Lynch et al., 1970; Keyl and Booth, 1971; Lynch, 1972). The toxic and carcinogenic M_1 form, which results from conversion of AFB_1 by hepatic metabolism, can be secreted into milk (Allcroft and Carnaghan, 1963). Due to the high amount of milk and milk products consumed by humans, keeping the concentration of aflatoxin M_1 (**AFM_1**) in milk within safe levels is critical. To avoid the risk of aflatoxin ingestion and intoxication, agencies around the world have established acceptable limits for aflatoxin concentration in milk and feeds. In the United States, the Food and Drug Administration (**FDA**) stipulated action levels for aflatoxin in raw milk and lactating cow feeds are 0.5 and 20 μg/kg, respectively (FDA, 2000). The maximum allowable concentration set by the European Commission is 0.05 μg/kg of milk (EFSA, 2004). The goal of keeping feed and milk aflatoxin concentration below these limits can be difficult to achieve because mycotoxin-producing molds infect crops and grains before and after harvesting (Lopez-Garcia et al., 1999; Gonzáles Pereyra et al., 2008; Richard et al., 2009). Furthermore, damage from insects, hail, lodging, and diseases can predispose plants to mycotoxin contamination (Council for Agricultural Science and Technology; CAST, 2003; Queiroz et al., 2012).

Many postharvest treatments are used for detoxifying feeds contaminated with mycotoxins, including thermal inactivation, irradiation, fermentation, ammoniation, and nixtamalization (Lopez-Garcia et al., 1999; Jouany,

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2007). Most of these methods are costly, time consuming, or partially effective (Kutz et al., 2009), and most are impractical for detoxification of the large quantities of feed used by many US dairies. Ammoniation, the most commonly used postharvest treatment, only partially eliminates aflatoxin from the contaminated matrix (Weng et al., 1994) and it can combine with sugars in forages under high temperatures to produce 4-methyl imidazole, which causes hyperexcitability in cattle (Kerr et al., 1987). Studies have demonstrated that dietary addition of adsorbent clays is a promising and effective way to prevent aflatoxin intoxication by livestock on farms (Stroud, 2006). However, few studies have examined the effect of the clay dose on efficacy in reducing AFM_1 carryover into the milk of dairy cows. Even fewer studies have examined dose effects of AFB_1 on the immune response in dairy cows, even though it is known to be immunotoxic or immunosuppressive in other species (Reddy and Sharma, 1989; Qureshi et al., 1998).

The objectives of this study were to determine the effect of adding 2 doses of a commercial montmorillonite hydrated sodium-calcium-aluminosilicate clay-based mycotoxin adsorbent on milk AFM_1 concentrations and the performance and immune response of dairy cows fed a diet contaminated with AFB_1.

MATERIALS AND METHODS

Cows, Treatments, and Design

Animals used in this study were cared for according to protocols approved by the University of Florida Institute of Food and Agricultural Sciences Animal Research Council (Gainesville). Eight lactating Holstein cows in late lactation (295 ± 45 DIM; mean ± SD) were stratified by milk production and randomly assigned to 1 of 4 treatments arranged in a balanced, replicated 4 × 4 Latin square design. Cows were housed in an open-sided, freestall barn bedded with sand and equipped with Calan gates (American Calan Inc., Northwood, NH) for individual feeding and misters and fans to minimize heat stress. The following treatments were investigated: (1) control diet (**C**), (2) aflatoxin diet (**T**) containing C and 75 μg/kg of AFB_1 in the TMR, (3) low-clay (**LC**) diet containing T and the adsorbent added at 0.2% of the TMR DM, and (4) high-clay (**HC**) diet containing T and the adsorbent added at 1% of the TMR DM. The clay adsorbent was Calibrin A (Amlan International, Chicago, IL) and the manufacturer specified the doses tested. Mycotoxin adsorbents have been added to diets at rates of 0.05 to 2% of the DM (Diaz et al., 2003; Whitlow and Hagler, 2005a).

Diets were formulated to meet or exceed the nutrient requirements of Holstein cows in late lactation producing 20 kg of milk/d (NRC, 2001). The ingredient and chemical compositions of the basal TMR fed to all cows are shown in Table 1. Cows were fed the toxin and adsorbent based on an estimated average DMI of 23 kg/d, resulting in a daily intake of 1,725 μg of AFB_1 for T, LC, and HC treatments, and 46 and 230 g of adsorbent in the LC and HC diets, respectively. Dietary AFB_1 was obtained from an *Aspergillus parasiticus* (NRRL-2999) culture at the University of Missouri Diagnostic Laboratory (Columbia). It contained 640 mg of AFB_1/kg, 22 mg of aflatoxin B_2/kg, 333 mg of aflatoxin G_1/kg, and 3 mg of aflatoxin G_2/kg. Both doses of the adsorbent were mixed into the respective TMR and fed daily to cows on LC and HC treatments. The daily dose of AFB_1 was divided into 2 portions and each was mixed with 20 mL of molasses and 400 g of corn silage to facilitate consumption. The mixture was fed to cows in a plastic container before the rest of the TMR was fed at the a.m. and p.m. feeding times (0700 and 1700 h). The AFB_1 was only added to diets on d 6 to 9 of each period. Days 1 to 5 were for adaptation to the binder, d 6 to 9 for dosing the toxin, and d 10 to 12 for clearance of the toxin from the cow's milk. Studies of Frobish et al. (1986) and Diaz et al. (2004) confirmed the appropriateness of the durations of the dosing and clearance periods. The 5-d adaptation period also reduced the risk of carryover of toxin effects from the previous period to the next one. Intake of the TMR was restricted to 95% of that in a 2-wk pretrial period when a common diet was fed to all cows to ensure that the adsorbent was completely consumed. The toxin was fed before the rest of the TMR to ensure it was completely consumed and to prevent contamination of equipment and the feed bunk with the toxin.

Experimental Measurements and Analytical Procedures

Cows were milked twice per day at 0100 and 1300 h and milk weights were recorded. Two milk samples were collected from a.m. and p.m. milkings on d 5, 9, 10, 11, and 12 in each period. Samples were analyzed by Southeast Dairy laboratories (Belleview, FL) for fat, protein, and SCC using a Bentley 2000 near-infrared reflectance spectrophotometer (Bentley Instruments Inc., Chaska, MN). Somatic cell scores were generated as described by Norman et al. (2000) for statistical analysis of SCC. Values for 3.5% FCM yield were calculated according to the equation [(0.4324 × milk yield) + (16.218 × milk fat yield)] (NRC, 2001). Milk AFM_1 concentration was quantified using the RIA test (CHARM II test; Charm Sciences Inc., Maiden, MA) described by Diaz et al. (2004), which had been validated against HPLC measurements in a ring test (Salter et al., 2006).

Table 1. Ingredient and chemical composition of the experimental diet

Item	Amount
Ingredient composition (% of DM)	
Corn silage	40.9
Alfalfa hay	8.05
Wet brewers grains	5.56
Distillers grains	6.97
Dried citrus pulp	3.34
Ground corn	18.2
SoyPlus[1]	4.03
Soybean meal	5.26
Sugarcane molasses	3.95
Mineral and vitamin mix[2]	3.74
Chemical composition	
DM (%)	46.7
Ash (% of DM)	5.8
CP (% of DM)	15.5
NDF (% of DM)	39.1
ADF (% of DM)	20.4
Aflatoxin B_1 (μg/kg)	ND[3]
Aflatoxin B_2 (μg/kg)	ND
Aflatoxin G_1 (μg/kg)	ND
Aflatoxin G_2 (μg/kg)	ND
Deoxynivalenol (mg/kg)	ND
T-2 toxin (mg/kg)	ND
Zearalenone (mg/kg)	ND

[1]West Central Soy, Ralston, IA.

[2]The mineral mix contained 23.7% CP, 9.7% Ca, 8.0% Na, 6.7% K, 2.4% Mg, 0.4% S, 0.9% P, 2,886 mg of Mn/kg, 3,092 mg of Zn/kg, 886 mg of Cu/kg, 339 mg of Fe/kg, 31 mg of Co/kg, 30 mg of I/kg, 17.0 mg of Se/kg, 147,756 IU of vitamin A/kg, and 787 IU of vitamin E/kg (DM basis).

[3]ND = concentrations of respective toxins were below lower detection limits (5 μg/kg for aflatoxins, and 0.5 mg/kg for deoxynivalenol, T-2 toxin, and zearalenone).

Weights of feed offered and refused by each cow were recorded daily. Dietary ingredients (corn silage, alfalfa hay, and concentrate mix) were sampled representatively daily. Four composites of the daily samples in each period were subsampled, dried at 60°C for 48 h in a forced-air oven, ground to pass the 1-mm screen of a Wiley mill (A. H. Thomas Co., Philadelphia, PA), and analyzed for DM (105°C for 16 h) and ash (512°C for 8 h) content. Concentrations of NDF and ADF were measured using the nonsequential method of Van Soest et al. (1991) in an Ankom 200 Fiber Analyzer (Ankom Technologies Corp., Macedon, NY). Heat-stable amylase was used in the NDF assay but sodium sulfite was not. Nitrogen was determined by rapid combustion using a Macro elemental N analyzer (Vario MAX CN, model ID 25.00-5003; Elementar Analysensysteme GmbH, Hanau, Germany) and CP was calculated as N × 6.25. The concentrations of aflatoxin B_1, B_2, G_1, and G_2, deoxynivalenol, T-2 toxin, and zearalenone in the ingredients were determined at University of Missouri Diagnostic Laboratory using the HPLC method described by Kutz et al. (2009).

Blood samples (10 mL) were collected on d 5 and 9 of each period from the coccygeal vein into Vacutainer tubes containing sodium heparin anticoagulant (Becton, Dickinson and Co., Franklin Lakes, NJ), stored on ice during transport, centrifuged at 2,500 × *g* for 20 min at 4°C to separate plasma, and stored at −20°C until analyzed. Plasma haptoglobin concentrations were determined by measuring haptoglobin/hemoglobin complexing based on differences in peroxidase activity (Makimura and Suzuki, 1982). Plasma ceruloplasmin oxidase activity was measured using the colorimetric procedure described by Demetriou et al. (1974). Plasma fibrinogen concentrations were determined using a kit (Sigma procedure no. 880; Sigma Diagnostics, St. Louis, MO) as described by Arthington et al. (2003). Neutrophil phagocytic activity was measured by monitoring the uptake of *Escherichia coli* particles labeled with a pH-sensitive dye from pHrodo *E. coli* BioParticles Conjugate (Invitrogen Life Sciences, Carlsbad, CA). Briefly, 40 μL of pHrodo *E. coli* was added to a 100-μL aliquot of blood containing no more than 5×10^3 cell/μL. The solution was incubated for 2 h at 37°C and cell membranes were disrupted by incubation in 2.5 mL of lysis buffer for 15 min at room temperature (20°C). The suspension was centrifuged at 2,000 × *g* for 5 min and washed with fluorescence-activated cell sorting buffer before a final centrifugation at 2,000 × *g* for 5 min. The pellet was kept on ice and phagocytotic activity was measured using a flow cytometer with a 488-nm excitation wavelength (FACSort; Becton Dickinson, San Jose, CA). Neutrophil adhesion molecules, CD62 (L-selectin) and CD18 (β_2-integrin), were quantified by flow cytometry as described by Silvestre et al. (2011), and the antibodies were obtained from AbD Serotec (Raleigh, NC).

Calculations

Aflatoxin M_1 secretion was calculated as the product of milk AFM_1 concentration on d 9 and milk yield on d 9; transfer of the toxin to milk was calculated as follows: (secretion of AFM_1 on d 9/daily AFB_1 intake); reduction of AFM_1 excretion by a specific treatment was defined as the percentage difference between the amount of milk AFM_1 excretion from treatment T and that for the treatment in question on d 9. Secretion, carryover, and reduction data were calculated using d 9 data (the fourth day of dosing) because up to 4 d are needed to achieve the steady state of milk AFM_1 concentration in cows fed AFB_1 (Polan et al., 1974; Diaz et al., 2004). Aflatoxin clearance rate was estimated as the slope of the line fitted through points, reflecting the decrease in milk AFM_1 concentration over time from d 10 to 12.

Table 2. Effect of dietary addition of aflatoxin B_1 (AFB_1) with or without low (LC) or high (HC) doses of a mycotoxin binder[1] on the performance of dairy cows during the aflatoxin dosing period (d 6 to 9)

Item	Control	AFB_1 toxin	LC + T[2]	HC + T	SEM
DMI (kg/d)	20.3	18.0	18.1	20.4	1.24
Milk yield (kg/d)	19.5	18.9	19.9	19.1	1.11
3.5% FCM (kg/d)	20.8	19.0	20.5	19.4	0.79
Milk protein (%)	3.36[ab]	3.28[c]	3.35[b]	3.41[a]	0.095
Milk fat (%)	3.75	3.78	3.68	3.69	0.180
Milk protein (kg/d)	0.65	0.62	0.67	0.65	0.05
Milk fat (kg/d)	0.74[a]	0.67[b]	0.73[ab]	0.69[ab]	0.03
SCC (×1,000/mL)	272	147	194	260	112

[a–c]Means within a row with no common superscript differ significantly ($P < 0.05$).

[1]Produced by Amlan International, Chicago, IL.

[2]T = aflatoxin diet.

Statistical Analysis

The MIXED procedure of SAS (version 9.2; SAS Institute Inc., Cary, NC) was used to analyze the data. The model for analyzing the data included treatment, period, square, treatment × period, and treatment × square interactions, and cow within square as the random effect. Data from blood and milk samples collected on d 5 were used as covariates for analyzing data obtained during the toxin-dosing period. Blood data were not normally distributed; therefore, they were log-transformed and analyzed with the GLIMMIX procedure of SAS. Fisher's F-protected least significant difference test was used to compare least squares means. Significance was declared at $P \leq 0.05$ and tendencies were declared if $P > 0.05 \leq 0.10$.

RESULTS AND DISCUSSION

Dry matter intake and milk yield were not affected by treatment ($P > 0.05$) and averaged 19.2 and 19.4 kg/d, respectively (Table 2). Feeding diet T instead of C tended to reduce 3.5% FCM yield (19.0 vs. 20.8 kg/d, $P = 0.08$). Kutz et al. (2009) detected no changes in DMI or milk yield when 112 μg of AFB_1/kg of TMR DM was fed to dairy cows in early to midlactation for 7 d. Stroud (2006) reported no changes in milk yield due to feeding 170 μg of AFB_1/kg of TMR DM for 11 d, but the toxin decreased DMI by 1.5 kg of DM/d compared with the DMI of cows not supplemented with AFB_1. Whitlow and Hagler (2005b) suggested that diets contaminated with aflatoxin levels above 100 μg/kg of diet may adversely affect animal performance and health; therefore, it is noteworthy that feeding 75 μg/kg of the toxin in this study also tended to reduce 3.5% FCM yield. Stroud (2006) noted that dosing cows with AFB_1 concentrations less than 150 μg/kg is more common than using higher rates but a dose of 50 μg/kg is sufficient to exceed the FDA action limit of 0.5 μg/kg AFM_1 in milk, assuming transfer of 1.0% into milk.

Compared with diet C, diet T reduced milk protein concentration (3.28 vs. 3.36%, $P = 0.01$) and milk fat yield (0.67 vs. 0.74 kg/d, $P = 0.04$). Aflatoxin can bind to some fractions of milk protein (Barbiroli et al., 2007) but the reduction in milk protein concentration is probably more related to the fact that aflatoxin directly inhibits protein synthesis (Garvican et al., 1973). However, Smith et al. (1994) did not detect changes in milk protein and fat percentages due to feeding 200 μg of AFB_1/kg of diet DM to goats. Kutz et al. (2009) reported that milk protein and fat percentage were unaffected when dairy cows were fed an aflatoxin-contaminated diet. The reason why the toxin adversely affected milk quality in the current study but not others may be partly due to the combination of different aflatoxins fed in the current study. Natural dietary mycotoxin contamination of diets typically results in more adverse effects on cows than purified mycotoxins because of synergistic effects of different toxins (Applebaum et al., 1982). The similarity in milk quality and 3.5% FCM yield measures among diets C, LC, and HC indicate that both doses of the adsorbent prevented adverse effects of the toxin on milk production and composition.

On the final day of dosing AFB_1 (d 9), concentrations of AFM_1 in milk from cows fed diets T, LC, and HC (0.57, 0.64, and 0.46 μg/kg, respectively) were greater ($P < 0.05$) than those of cows fed diet C (0 μg/kg; Table 3) and concentrations were lower ($P < 0.05$) in cows fed HC than those receiving diets T or LC. Relative to diet T, diet HC reduced the milk AFM_1 concentration by 17% but diet LC did not reduce the value ($P > 0.05$). Milk AFM_1 concentrations were greater than the FDA action level (0.5 μg/kg) in cows fed T or LC, whereas those of cows fed HC were lower. Other studies have also reported that mycotoxin binders reduced the AFM_1 concentration of milk. Diaz et al. (2004) compared effects of adding activated carbon, esterified glucomannan, calcium bentonite, and 3 sodium bentonite products at 1.2% of diet DM on concentrations

Table 3. Effect of dietary addition of aflatoxin B_1 (AFB_1) with or without low (LC) or high (HC) doses of a mycotoxin binder[1] on the aflatoxin M_1 (AFM_1) concentration in the milk

Item	AFB_1 toxin	LC + T[2]	HC + T	SEM
Concentration on d 9[3] (μg/kg)	0.57[a]	0.64[a]	0.46[b]	0.04
Secretion on d 9 (μg/d)	10.6[ab]	13.0[a]	8.8[b]	1.55
Transfer on d 9 (%)	0.61[ab]	0.75[a]	0.51[b]	0.05
Reduction in secretion on d 9 (%)	0[c]	−22[c]	16[b]	7.05
Clearance d 10–12 (μg/h)	0.011[ab]	0.013[a]	0.009[b]	0.0008

[a–c]Means within a row with no common superscript differ significantly ($P < 0.05$).

[1]Produced by Amlan International, Chicago, IL.

[2]T = aflatoxin diet.

[3]Day 9 was the last of 4 d of dosing the toxin, which typically reflects the time when AFM_1 concentrations in milk achieve steady state in cows fed AFB_1.

of AFM_1 in milk of cows fed diets contaminated with 100 μg of AFB/kg. Respective reductions in milk AFM_1 concentrations were 5.4, 59, 31, 65, 50, and 61%. Kutz et al. (2009) evaluated the effect of adding 2 hydrated-sodium-calcium aluminosilicates (Novasil Plus, Engelhard Corp., NJ, and Solis, Novus International Inc., St. Charles, MO) or an esterified glucomannan product (MTB-100; Alltech, Nicholasville, KY) at 0.5% of diet DM on concentrations of AFM_1 in milk of cows fed 100 μg of AFB_1/kg of diet. The aluminosilicate products reduced milk AFM_1 concentration by 45 and 48%; however, the esterified glucomannan caused a reduction of only 4%.

The effects of the strategy used to administer the toxin and adsorbent on performance of cows and milk AFM_1 concentration are unknown. In this study, the toxin was added to ingredients fed before the rest of the TMR was fed to ensure complete consumption of the toxin and minimize contamination of equipment. However, the approach may have limited binding of the toxin by the adsorbent if the outflow rate of the toxin from the rumen was more rapid than that of the digesta. Diaz et al. (2004) and Stroud (2006) mixed the toxin and adsorbent with the rest of the ingredients in the TMR. Although this approach facilitates binding of the toxin to the TMR, it results in incomplete ingestion of the toxin. Consequently, the recent study of Kutz et al. (2009) used a similar approach to that in the current study to ensure complete consumption of the toxin and to minimize equipment contamination and the associated health and safety risks.

Secretion and transfer of aflatoxin into the milk were lower in cows fed HC than those fed LC but cows fed T had similar values to those fed LC or HC. However, the percentage reduction in aflatoxin secretion relative to that in cows fed diet T was greater in cows fed HC than LC. The latter and the numerical trend for lower carryover and secretion of the toxin for diet HC than diets T or LC is consistent with the lower AFM_1 concentration in milk of cows fed HC than in those fed T or LC. The lower efficacy of the low versus high adsorbent application rate reflects the fact that adsorption of aflatoxin by aluminosilicate binders happens in a dose-dependent manner (Sarr, 1995). Even though application rates of mycotoxin adsorbents to ruminant diets range from 0.5 to 1.2% of dietary DM (Harvey et al., 1991; Stroud, 2006; Kutz et al., 2009), low rates have not been effective consistently at mitigating effects of dietary aflatoxin on the performance, health and milk AFM_1 concentrations of dairy cows.

The carryover of dietary aflatoxin into milk was reported to be between 0 and 4% by Sieber and Blanc (1978, as described by Van Egmond, 1989) and between 1 and 6% by the European Food Safety Authority (EFSA, 2004). Lafont et al. (1980) fed daily doses of 0.09, 0.18, 0.86, or 2.58 mg to cows in early and late lactation producing 20 L of milk/d, on average, and the respective carryovers were 0.78 and 0.22%, respectively. The average milk production of the late-lactation cows in the current study was similar at 19.4 kg/d but their aflatoxin carryover was closer to the higher value in the Lafont et al. (1980) study. Frobish et al. (1986) reported that high-producing cows have greater carryover than cows with moderate-to-low milk production. Cows in the current study and that of Lafont et al. (1980) also had lower carryover than the mean value of 2% reported for cows producing 33.8 kg/d in a similar study (Kutz et al., 2009). Stroud (2006) reviewed 14 studies on effects of adsorbents on aflatoxin transfer and reported that the mean carryover was about 1% as in the current study, when diets were dosed with up to 150 μg of aflatoxin/kg.

The pattern of decrease in AFM_1 in the milk after toxin dosing was terminated on d 9 (clearance rate) is shown in Figure 1. No AFM_1 was detected in the milk of any cow by d 12. Clearance of the toxin within 3 d agrees with published data. Frobish et al. (1986) reported that after 3 to 4 d of AFB_1 removal from diets, the AFM_1 concentration in milk should be reduced to normal levels. The studies of Applebaum et al.

(1982) and Diaz et al. (2004) also confirmed that AFM_1 cleared from the milk of animals receiving an AFB_1-contaminated diet within 4 d. Masoero et al. (2007) detected that within 24 h of toxin withdrawal from the diet, milk AFM_1 levels dropped below the FDA legislative limit (0.5 μg/kg) and this decrease was significant ($P < 0.05$) within 48 h of withdrawing the toxin from the diet. In the current study, no AFM_1 was detected in the milk of cows fed diet HC 24 h after the toxin was withdrawn from the diet but low quantities were still detected in cows fed T and LC at that time, indicating that AFM_1 clearance was increased by HC but not LC.

Treatment effects on immune responses are summarized in Table 4. Haptoglobin concentrations in plasma were greater ($P < 0.01$) in cows fed diet T than other diets, whereas values for cows fed diets C, LC, and HC did not differ. Stimulation of the acute-phase response due to inflammatory stress is characterized by increased secretion of acute-phase proteins such as ceruloplasmin and haptoglobin (Bertoni et al., 2008). Haptoglobin is often used in ruminants as a biomarker to identify immune-challenged animals (Heegaard et al., 2000; Arthington et al., 2003). Hiss et al. (2004) demonstrated the sensitivity of haptoglobin to immune stressors by showing that blood haptoglobin concentration increased 11.3 fold after 12 h of intramammary administration of LPS. That blood haptoglobin concentrations were elevated when diet T was fed but not when diets C, LC, or HC were fed indicates that both doses of the adsorbent prevented the increased innate immune inflammatory stress response caused by the toxin. Fibrinogen and ceruloplasmin are also acute-phase protein markers of the innate immune response, yet their concentrations did not differ among treatments. This is partly because these markers are often less sensitive and respond less consistently than haptoglobin to inflammatory stressors (Arthington et al., 2003).

The fluorescence intensity observed when evaluating the activity of adhesion molecules indicates the pres-

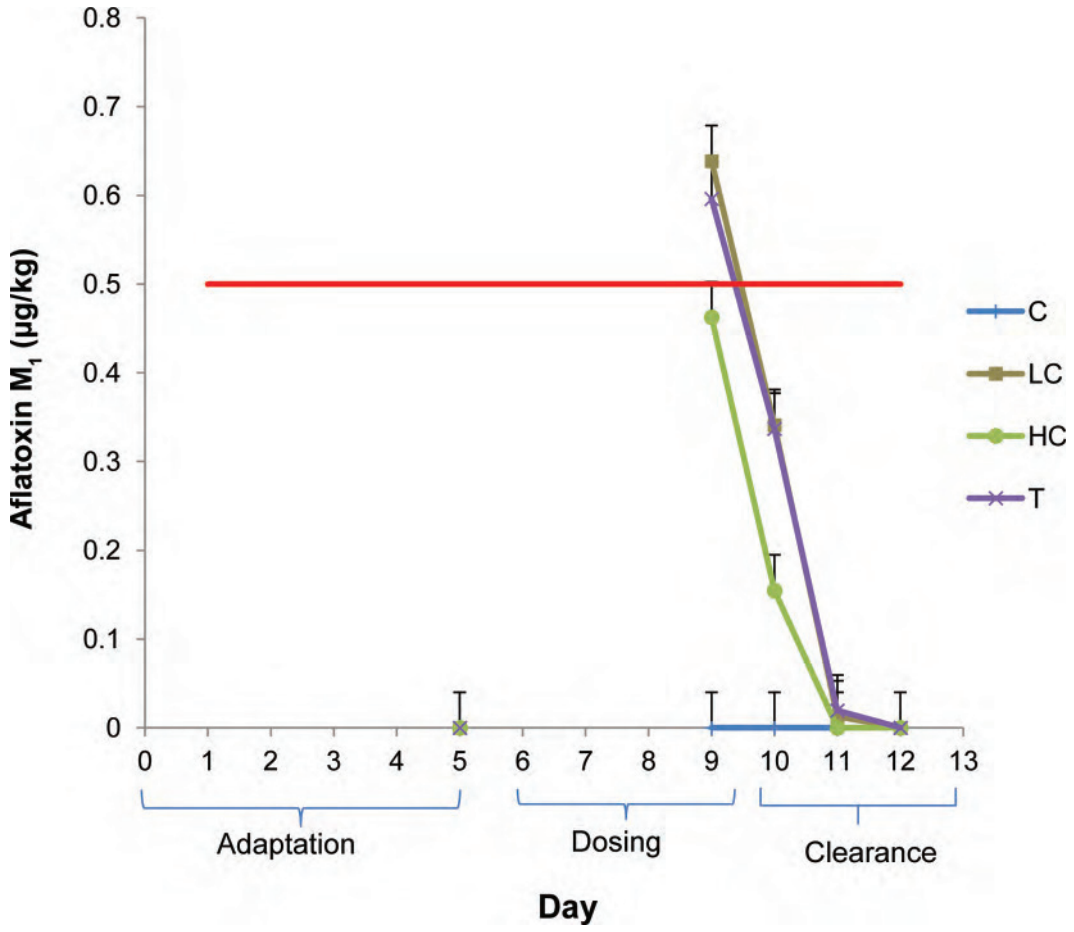

Figure 1. Effect of dietary addition of aflatoxin B_1 with or without low (LC) or high (HC) doses of a mycotoxin binder on clearance of aflatoxin M_1 (AFM_1) from the milk of dairy cows; C = control diet with no aflatoxin or binder; T = diet with 75 μg/kg of aflatoxin B_1 but without binder. The horizontal line indicates the Food and Drug Administration (FDA) action level. Error bars indicate standard errors. Color version available in the online PDF.

Table 4. Effect of dietary addition of aflatoxin B_1 (AFB_1) with or without low (LC) or high (HC) doses of a mycotoxin binder[1] on markers of the innate immune response in blood plasma on the last day (d 9)[2] of feeding AFB_1

Item	Control	AFB_1 toxin	LC + T	HC + T	SEM
Acute-phase proteins					
Ceruloplasmin (mg/100 mL)	21.0	21.5	20.2	22.2	1.18
Haptoglobin[3] (arbitrary unit)	14.4[b]	22.0[a]	14.8[b]	16.0[b]	1.98
Fibrinogen (mg/100 mL)	270	301	278	275	25
Median fluorescence intensity of neutrophils and neutrophil-adhesion molecules					
β_2-integrin (CD18)	131.1	219.7	154.7	138.2	32
L-selectin (CD62)	822.4	951.6	977.8	912.5	129
Neutrophils	100	113	110	102	14
Neutrophil phagocytosis (%)	82.6	79.6	82.2	83.0	3.0

[a,b]Means within a row with no common superscript differ significantly ($P < 0.05$).

[1]Produced by Amlan International, Chicago, IL.

[2]Day 9 was the last of 4 d of dosing the toxin, which typically reflects the time when AFM_1 concentrations in milk achieve steady state in cows fed AFB_1.

[3]Absorbance × 100 at 450 nm.

ence of receptors for β_2-integrin or L-selectin on the leukocyte membrane (Murphy et al., 2008). Both are adhesion molecules that facilitate the interaction between leukocytes and the endothelium that is needed to allow diapedesis, which is further cellular extravasation of leukocytes to the site of infection. In this study, feeding diet T instead of C tended to increase the expression of β_2-integrin (220 vs. 131, $P = 0.10$), which agrees with the haptoglobin response. Collectively, the response to haptoglobin and β_2-integrin indicate that the toxin increased inflammatory stress, but feeding either dose of the adsorbent prevented this problem.

Dietary treatments did not affect the percentage of phagocytotic neutrophils or neutrophil phagocytosis (Table 4). The technique used to evaluate neutrophil phagocytosis involved using *E. coli* cells stained with a pH-sensitive dye, which becomes fluorescent when bacterial cells are exposed to the acidic environment within the leukocyte phagosome. This technique is more convenient, rapid, and less laborious than more traditional techniques (Silvestre et al., 2011). However, it does not involve using dihydrorhodamine 123 or other oxidase-peroxidase markers; therefore, it cannot account for oxidative burst, which is the capacity of neutrophils to destroy the phagocytized bacteria.

CONCLUSIONS

Feeding AFB_1 did not affect DMI or milk yield but tended to decrease FCM yield, decreased milk fat yield and milk protein concentration, increased the innate immune response, and increased milk AFM_1 concentration to levels that exceeded the FDA legislative limit. The low and high doses of the mycotoxin adsorbent prevented the adverse effects of the toxin on the immune response, milk quality, and FCM yield but only the high dose decreased the AFM_1 concentration of the milk and increased the clearance rate.

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