Efficacy of Solis, NovasilPlus, and MTB-100 to reduce aflatoxin M₁ levels in milk of early to mid lactation dairy cows fed aflatoxin B₁

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ABSTRACT

An experiment was conducted to determine the efficacy of 3 adsorbents, Solis (SO; Novus International Inc.), NovasilPlus (NOV; Engelhard Corp.), and MTB-100 (MTB; Alltech), in reducing aflatoxin (AF) M₁ concentrations in milk of dairy cows fed an AF-contaminated diet. Twelve early to mid lactation dairy cows averaging 163 d in milk were used in a 4 × 4 Latin square design with 3 replications. Cows were blocked by parity, body weight, and milk production and were provided ad libitum access to feed and water. Within each replicate, cows were randomly assigned to the 4 dietary treatments for 4 consecutive 7-d periods. Dietary treatments included AF [112 µg of AFB₁/kg of diet dry matter (DM)]; AF + 0.56% SO; AF + 0.56% NOV; and AF + 0.56% MTB. Milk samples were collected on d 6 and 7 of each of the experimental periods. Feed intake, milk production, milk fat percentage, milk protein percentage, and linear somatic cell scores were not affected by dietary treatments and averaged 22.20 kg/d of DM, 33.87 kg/d, 3.78%, 2.95%, and 1.60, respectively, across all treatments. Transfer rates of AF from feed to milk averaged 2.65, 1.48, 1.42, and 2.52% for cows fed AF, AF + SO, AF + NOV, and AF + MTB, respectively. Daily AFM₁ excretion in milk averaged 66, 37, 35, and 63 µg/d for cows fed AF, AF + SO, AF + NOV, and AF + MTB, respectively. The addition of SO and NOV to the AF diet resulted in a significant reduction in milk AFM₁ concentrations (SO, 45%; NOV, 48%) and AFM₁ excretion (SO, 44%; NOV, 46%). In contrast, MTB was not effective in reducing milk AFM₁ concentrations (4%), AFM₁ excretion (5%), or AF transfer from feed to milk (2.52%). Results indicated that SO and NOV at 0.56% of the diet were effective in reducing milk AFM₁ concentrations in cows consuming a total mixed ration containing 112 µg of AFB₁/kg of diet DM.

Key words: aflatoxin B₁, aflatoxin M₁, lactating dairy cow, adsorbent

INTRODUCTION

Aflatoxins (AF), a class of mycotoxins produced by fungal species of the genus *Aspergillus* (*flavus* and *parasiticus*), are sometimes found in feed ingredients used in dairy rations. Major forms of aflatoxins include AFB₁, AFB₂, AFG₁, and AFG₂, with AFB₁ being the most common and biologically active component (Busby and Wogan, 1981). In general, dietary concentrations of AF found in dairy rations are not usually high enough to cause reductions in feed intake and milk production. However, at these lower dietary AF concentrations AFM₁, a metabolite of AFB₁, is secreted into the milk. Aflatoxin M₁ is toxic and carcinogenic, and is of great concern with respect to human health because of the high consumption of milk and milk products by humans, especially children. Because of this concern, the AFM₁ level in milk is regulated by the US Food and Drug Administration at a maximum of 0.5 µg/L.

Measures used by the livestock industry to protect animals from the toxic effects of AF include grain testing (Stoloff and Scott, 1984), use of mold inhibitors (Hamilton, 1985), fermentation (Dam et al., 1977), microbial inactivation (Ciegler et al., 1966), physical separation (Huff, 1980), thermal inactivation (Conway et al., 1978), irradiation (Shantha and Sreenivasa, 1977), ammoniation (Brekke et al., 1977, 1979), ozonation (McKenzie et al., 1997, 1998), and the use of adsorbents (Masimanco et al., 1973; Phillips et al., 1990a,b). Unfortunately, most of these measures are costly, time consuming, and only partially effective. At present, one of the more effective and practical approaches is the use of adsorbents. Selected adsorbents added to AF-contaminated feeds can sequester AF during the digestive process, allowing AF to pass harmlessly through the animal (Davidson et al., 1987; Phillips et al., 1990a,b). Major advantages of these adsorbents are that they are relatively inexpensive, generally recognized as safe (GRAS), and can be eas-

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ily added to animal feeds. However, not all adsorbents are equally effective at sequestering AF (Stroud, 2006). An experimental lactating dairy cow model has been developed (Diaz et al., 2004; Stroud, 2006) to evaluate the effectiveness of different adsorbents at reducing AFM₁ levels in milk. Using this model, we hypothesized that different sources of adsorbents would differ in their effectiveness to sequester AF in feed and, consequently, to reduce AFM₁ in milk. It should be noted that none of these products have been approved for commercial use by FDA. The objective of this study was to determine the efficacy of 3 adsorbents, Solis (Novus International Inc., St. Charles, MO), NovasilPlus (Engelhard Corp., Cleveland, OH), and MTB-100 (Alltech Inc., Nicholasville, KY), to reduce the levels of AFM₁ in the milk of early to mid lactating dairy cows fed AF.

MATERIALS AND METHODS

Experimental Procedures

Twelve early to mid lactation dairy cows averaging 651 ± 62 kg of BW and 163 ± 54 DIM were transported from the University of Missouri Dairy Research Center and housed in Unit C of the Animal Sciences Research Center. Cows were provided ad libitum access to feed and water. During the study, all cows were treated with Posilac (recombinant bST, Elanco Animal Health, Indianapolis, IN) every 2 wk according to the manufacturer's recommendations. The experimental design consisted of a 4×4 Latin square replicated 3 times. Cows were blocked by parity, BW, and milk production. Each block was housed in a separate environmentally controlled room. Cows were assigned to a randomized schedule to receive each of the 4 dietary treatments during 4 consecutive 7-d periods.

The dietary treatments were formulated to include: A) 100 µg of AFB₁/kg of diet DM; B) 100 µg of AFB₁/kg of diet DM + 0.5% Solis; C) 100 µg of AFB₁/kg of diet DM + 0.5% NovasilPlus; and D) 100 µg of AFB₁/kg of diet DM + 0.5% MTB-100. Both NovasilPlus and Solis are classified as hydrated sodium calcium aluminosilicates (**HSCAS**). By contrast, MTB-100 is a commercial modified yeast cell culture preparation based on a *Saccharomyces cerevisiae* strain 1026, with HSCAS as one of the stated ingredients on the label.

Each period of the Latin square lasted 7 d, with milk samples collected on d 6 and 7 for analysis of milk AFM₁ concentrations. Dietary AFB₁ was supplied by *Aspergillus parasiticus* (NRRL-2999) culture material containing 760 mg/kg of AFB₁, 28 mg/kg of AFB₂, 440 mg/kg of AFG₁, and 13 mg/kg of AFG₂. The TMR fed to all cows in the study consisted of corn silage, alfalfa silage, alfalfa hay, ground corn, soybean meal,

Table 1. Ingredient and nutrient composition of the TMR fed to lactating dairy cows

Item	Amount (% of diet DM)
Ingredient	
Corn silage	23.8
Alfalfa silage	9.8
Alfalfa hay	13.4
Ground corn	22.4
Soybean meal (48%)	1.7
Whole cottonseed	12.1
Wet brewers grains	1.7
Roasted soybeans	4.4
High premix	3.6
Brome hay	1.7
Soyhulls	5.3
Nutrient	
DM	54.6
CP	16.9
ADF	24.0
NDF	35.3

whole cottonseed, wet brewers grain, roasted soybeans, brome hay, soyhulls, and mineral and vitamin premixes. The diet was formulated to meet the nutrient requirements of a second-lactation Holstein producing 41 kg/d of milk containing 3.8% milk fat, as defined by NRC (2001; Table 1).

It was estimated that cows would consume an average of 25 kg of DM each day, resulting in a daily intake of 2,500 µg of AFB₁ (25 kg of DM $\times$ 100 µg of AFB₁/kg) and a daily intake of 125 g (25 kg of DM $\times$ 0.5%) of adsorbent. Cows were fed the TMR in 3 equal aliquots daily (0700, 1200, and 1900 h). The daily dose of AFB₁ was divided into 3 aliquots, and each aliquot was mixed with 99 g of grain containing molasses to encourage consumption. The daily dose of adsorbents was also divided into 3 aliquots and each aliquot was mixed with 258 g of TMR. At each feeding period, cows were first fed the aliquots of AFB₁ in the grain mix, followed by the aliquots of adsorbent and TMR. After the cows consumed the AFB₁ + grain mix and the adsorbents + TMR mix (10 to 15 min), they were then fed one-third of their daily aliquot of TMR. This feeding regimen ensured that each cow consumed the allotted amounts of AFB₁ and adsorbent, and prevented AFB₁ contamination of TMR mixing facilities.

Analytical Procedures

A bronopol preservative (Broad Spectrum Microtabs II, D and F Control Systems Inc., Dublin, CA) was added to each milk sample and samples were sent to the Missouri DHIA laboratory (Springfield, MO) for analysis of milk components. Milk samples were collected on d 6 and 7 of each of the 4 experimental periods. On each of the 2 d, milk samples from the a.m. and p.m. milking were composited and frozen until analyzed.

Table 2. Effects of dietary treatments on milk production and composition of dairy cows fed 112 µg of aflatoxin B₁ (AFB₁)/kg of DM¹

Item	Milk (kg)	FCM ² (kg)	Fat (%)	Protein (%)	Linear SCS
Treatment ³					
AFB ₁	34.19	33.40	3.87	2.98	1.68
AFB ₁ + SO	34.13	32.97	3.81	2.95	1.66
AFB ₁ + NOV	33.73	32.29	3.75	2.87	1.16
AFB ₁ + MTB	33.43	31.76	3.67	2.99	1.89
Pooled SEM	0.38	0.78	0.12	0.05	0.47
Source of variation			P-value		
Cow	<0.0001	<0.0001	0.0016	0.0112	0.0208
Period	<0.0001	0.0007	0.4790	0.8528	0.9838
Treatment	0.4705	0.4696	0.7000	0.3205	0.7236

¹Data are means of 12 cows per treatment.²4% FCM (NRC, 2001).³SO = Solis (Novus International Inc., St. Charles, MO); NOV = NovasilPlus (Engelhard Corp., Cleveland, OH); MTB = MTB-100 (Alltech Inc., Nicholasville, KY).

Milk samples were thawed and centrifuged at $1,875 \times g$, and as much fat as possible was removed. The milk filtrates (25 mL) were then passed through AFLAPREP M immunoaffinity cleanup columns (R-Biopharm Rhone Ltd., Glasgow, Scotland). Columns were washed twice with 10 mL of PBS, and AFM₁ was then eluted from the columns with 1.5 mL of acetonitrile, followed by 1.5 mL of water. These were combined, placed in autosample vials, and analyzed by HPLC with fluorescence detection. The HPLC system consisted of a Hitachi Model L-7100 pump with a Hitachi Model L-7485 fluorescence detector (365 nm excitation and 440 nm emission), a Hitachi Model L-7200 autosampler with Hitachi D-7000 data acquisition interface, and Concert Chrom software on a microcomputer. The HPLC column was a 150×4.6 mm reversed-phase HyperClone 3-µm C₁₈ BDS column (Phenomenex) with a C₁₈ SecurityGuard precolumn (Phenomenex). The mobile phase was acetonitrile:methanol:water (15:15:70) and run at 1 mL/min. The injection volume was 50 µL for all standards and samples. The detection limit was set at 40 ng/kg of AFM₁.

Statistical Procedures

Data were analyzed by ANOVA using the MIXED procedure (SAS Institute, 2003) as a replicated Latin square split plot in time (repeated-measures ANOVA), with cow as the experimental unit (Littell et al., 1998). The linear statistical model contained the effects of cow, period, treatment, cow (period treatment), time, and time interaction of treatment $\times$ time. Cow (period treatment) was used as the denominator of *F* to test the main-plot effect. The residual mean was used as a denominator of *F* to test the day and the treatment $\times$ day interaction. Mean differences were tested using Fisher's least significant difference. All statements of significance are based on the 0.05 level of probability.

RESULTS AND DISCUSSION

It was estimated that cows would consume an average of 25 kg of DM each day, resulting in a daily intake of 2,500 µg of AFB₁ (25 kg of DM $\times$ 100 µg of AFB₁/kg), and a daily intake of 125 g (25 kg of DM $\times$ 0.5%) of adsorbent. However, daily DM feed intake averaged only 22.29 kg/cow, which resulted in a daily intake of 2,500 µg of AFB₁ but a dietary concentration of 112.2 µg of AFB₁/kg of DM. Similarly, the dietary concentration of the adsorbents changed from 0.5 to 0.56%.

With the exception of 1 cow that developed mastitis, cows did not behave abnormally and had no clinical signs associated with aflatoxicosis. Feed intake and milk production were not affected by dietary treatments ($P > 0.05$) and averaged 22.29 kg and 33.87 kg/d (Tables 2 and 3), respectively, across all treatments. Milk composition (Table 2) was also not affected ($P > 0.05$) by dietary treatments, with fat percentage, protein percentage, and linear SCS averaging 3.78%, 2.95%, and 1.60, respectively, across all treatments.

Effects of dietary treatments on AFM₁ residues in milk are summarized in Table 3. Aflatoxin M₁ concentrations for the control (no adsorbent), Solis, NovasilPlus, and MTB-100 treatments averaged 1.92, 1.06, 1.00, and 1.84 µg/kg, respectively. Compared with the AF-only diet, AFM₁ concentrations in milk were reduced ($P < 0.0001$) by the addition of Solis and NovasilPlus. The addition of MTB-100 to the AF diet was not effective in reducing milk AFM₁ concentrations ($P > 0.05$).

Aflatoxin M₁ excretion via milk, as calculated from milk AFM₁ concentration and total milk volume produced (concentration of AFM₁ in milk $\times$ amount of milk produced), was 66.21, 36.99, 35.47, and 62.98 µg/d for the control (no adsorbent), Solis, NovasilPlus, and MTB-100 treatments, respectively (Table 3). Compared with the AF-only diet, AFM₁ excretion in milk was reduced ($P < 0.0001$) by the addition of Solis and

Table 3. Efficacy of adsorbents in reducing aflatoxin M₁ (AFM₁) residues in milk of dairy cows fed 112 µg of AFB₁/kg of DMI¹

Item	DMI (kg/d)	AFM ₁ (µg/L)	Excretion (µg/d)	Transfer (%)
Treatment				
AFB ₁	22.16	1.92 ^a	66.21 ^a	2.65 ^a
AFB ₁ + SO (base)	22.57	1.06 ^b	36.99 ^b	1.48 ^b
AFB ₁ + NOV	22.25	1.00 ^b	35.47 ^b	1.42 ^b
AFB ₁ + MTB	22.16	1.84 ^a	62.98 ^a	2.52 ^a
Pooled SEM	0.19	0.11	3.93	0.16
Source of variation		<i>P</i> -value		
Cow	<0.0001	<0.0001	<0.0001	<0.0001
Period	<0.0001	0.5931	0.1874	0.1874
Treatment	0.3690	<0.0001	<0.0001	<0.0001

^{a,b}Values within columns with no common superscript differ significantly ($P < 0.05$).

¹Data are means of 12 cows per treatment. AFM₁ excretion = concentration of AFM₁ in milk × amount of milk produced; AF transfer = excretion of AFM₁ divided by aflatoxin B₁ (AFB₁) consumption (× 100); SO = Solis (Novus International Inc., St. Charles, MO); NOV = NovasilPlus (Engelhard Corp., Cleveland, OH); MTB = MTB-100 (Alltech Inc., Nicholasville, KY).

NovasilPlus. The addition of MTB-100 to the AF diet was not effective ($P > 0.05$) in reducing AFM₁ excretion in milk.

Transfer of AF from feed to milk, as calculated from AF intake and total milk volume [(excretion of AFM₁/AFB₁ consumption) × 100] averaged 2.65, 1.48, 1.42, and 2.52% for the control (no adsorbent), Solis, NovasilPlus, and MTB-100 treatments, respectively (Table 3). Compared with the AF-only diet, AFM₁ transfer from feed to milk was reduced ($P < 0.0001$) by the addition of Solis and NovasilPlus. The addition of MTB-100 to the AF diet was not effective ($P > 0.05$) in reducing AF transfer from feed into milk.

Milk AFM₁ concentrations ranged from 1.00 to 1.92 µg/L, and transfer rates of AF from feed to milk ranged from 1.42 to 2.65%. Transfer rates observed in the current study are consistent with previous reports for dairy cows indicating AF transference rates ranging from 0.25 to 4.8% (Applebaum et al., 1982; Price et al., 1985; Frobish et al., 1986; Harvey et al., 1991; Veldman et al., 1992; Stroud, 2006).

The addition of NovasilPlus (0.56%) to the AF diet resulted in a 48% reduction in milk AFM₁ concentrations, a 46% reduction in AF excretion, and a 47% reduction in AF transfer from feed to milk. Similar reductions in milk AFM₁ concentrations, AF excretion, and AF transfer in response to supplemental NovasilPlus have been reported previously. Harvey et al. (1991) reported a 44% reduction in milk AFM₁ concentrations of dairy cows fed 100 µg of AF/kg of feed and 1% NovasilPlus. More recently, Stroud (2006) reported a 40% reduction in milk AFM₁ concentrations, a 43% reduction in AF excretion, and a 42% reduction in AF transfer from feed to milk in dairy cows fed 170 µg of AFB₁/kg of feed and 0.5% NovasilPlus. Results of the current study with respect to NovasilPlus are consistent with previ-

ous reports on the efficacy of this product in reducing AFM₁ levels in milk of cows fed AF.

Results of the current study indicate that Solis at 0.56% of the diet was just as effective as 0.56% NovasilPlus in reducing milk AFM₁ concentrations (45 vs. 48%), AF excretion (44 vs. 46%), and AF transfer (44 vs. 47%) from feed to milk. The effectiveness of Solis has also been observed in growing broilers exposed to feed contaminated with AF (Shirley et al., 2008). However, this is the first report demonstrating the effectiveness of Solis in early to midlactating dairy cows. In contrast, MTB-100 (0.56%) was not effective in reducing AFM₁ concentrations (4%), AF excretion (5%), or AF transfer (5%) from feed to milk. Stroud (2006) also reported that MTB-100 at 0.5% of a diet containing 170 µg of AFB₁/kg of feed was not effective in reducing milk AFM₁ concentrations (−8%), AF excretion (−7%), or AF transfer (−4%) from feed to milk. Results of these 2 studies with MTB-100 are in contrast to a previous study (Diaz et al., 2004) in which MTB-100 at 0.05% of a diet containing 55 µg of AFB₁/kg was reported to reduce milk AFM₁ concentrations by 59%. The lower dietary concentration of AFB₁ (55 µg/kg of AFB₁) used in the study by Diaz et al. (2004) compared with that used in the current study (112 µg/kg of AFB₁) and in the study by Stroud (2006; 170 µg/kg of AFB₁) may have contributed to the observed differences among the studies.

The differences in milk AFM₁ reduction among the products tested might be due to their composition and mechanism of action of the active compounds. Solis and NovasilPlus are sources of HSCAS, whereas MTB-100 contains a modified yeast cell culture with some amount of HSCAS. Initially, Phillips et al. (1990a,b) proposed that AF was bound to HSCAS as a result of the β-carbonyl system of AF forming a complex with

uncoordinated edge site aluminum ions of HSCAS. It is now postulated that the major site of chemisorption of AF to HSCAS is at the interlayer surfaces (Phillips, 1999). The dicarbonyl portion of the AF molecule was found to be essential for tight binding of the molecule by HSCAS. The interaction with HSCAS makes AF unavailable for absorption.

In contrast, the mechanism for the modified yeast product has not been well described. Yiannikouris et al. (2003, 2004) proposed it as a 2-level interaction between the glucan portion of the yeast cell wall and the mycotoxin molecule. However, Yiannikouris et al. (2003, 2004) did not evaluate binding of AF, but instead evaluated binding of zearalenone to the yeast cell wall, and only under in vitro conditions.

CONCLUSIONS

Solis and NovasilPlus at 0.56% of the diet were effective in reducing milk AFM₁ concentrations in cows consuming a TMR containing 112 µg of AFB₁/kg of diet DM. Under the current study conditions, MTB-100 at 0.56% was not effective in reducing milk AFM₁ concentrations.

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