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Transfer of dietary aflatoxin B_1 to milk aflatoxin M_1 and effect of inclusion of adsorbent in the diet of dairy cows

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ABSTRACT

The objectives of this study were to investigate the transfer of aflatoxin from feed to milk and to evaluate the effects of Solis Mos (SM; Novus International Inc., St. Charles, MO) on milk aflatoxin M_1, plasma biochemical parameters, and ruminal fermentation of dairy cows fed varying doses of aflatoxin B_1 (AFB_1). Three groups of 8 multiparous Holstein cows in late lactation (days in milk = 271 ± 29; milk yield = 21.6 ± 3.1 kg/d) were assigned to 1 of 3 experiments in a crossover design. Cows in experiment 1 received no aflatoxin, cows in experiment 2 received 20 μg of AFB_1/kg of dry matter, and cows in experiment 3 received 40 μg of AFB_1/kg of dry matter. Cows in each experiment were assigned to 1 of 2 treatments: control or 0.25% SM. Each experiment consisted of 2 consecutive periods with the first 4 d (d 1 to 4) as adaptation, followed by AFB_1 challenge for 7 d (d 5 to 11), and finally clearance for 5 d (d 12 to 16) in each period. Samples of total mixed ration and milk were collected on d 1, 2, and 10 to 14 of each period. Blood samples were collected from the coccygeal vein on d 1, 11, and 14 of each period. Rumen fluid was collected by oral stomach tube 2 h after the morning feeding on d 1 and 11 of each period. Adding SM to basal or AFB_1-contaminated diets at 0.25% had no effect on lactation performance, liver function, or immune response. However, addition of SM improved antioxidative status, as indicated by increased plasma concentrations of superoxide dismutase and reduced malondialdehyde regardless of dietary AFB_1 level. Addition of SM to the AFB_1-free diet eliminated the background AFM_1 in milk and increased total ruminal volatile fatty acid (99.6 vs. 94.2 m*M*) concentrations. Adding SM to the AFB_1-contaminated diet in experiment 2 decreased the AFM_1 concentration (88.4 vs. 105.3 ng/L) and the transfer of aflatoxin to milk (0.46 vs. 0.56%), and increased total volatile fatty acid concentration (99.8 vs. 93.4 m*M*). Adding SM to diets with 40 μg/kg of AFB_1 did not elicit changes in rumen parameters or AFM_1 output. These results indicated that adding SM to diets containing 0 or 20 μg of AFB_1/kg decreased milk AFM_1 concentration, improved antioxidative status, and altered rumen fermentation, whereas adding SM to a diet containing 40 μg of AFB_1/kg did not reduce AFB_1 transfer but did increase the antioxidant status of the liver.

Key words: adsorbent, aflatoxin B_1, aflatoxin M_1, dairy cows, transfer

INTRODUCTION

Aflatoxins are secondary metabolites, produced by several species of the genus *Aspergillus*—*A. flavus*, *A. parasiticus*, *A. bombycis*, *A. ochraceoroseus*, *A. nomius*, and *A. pseudotamari*—that contaminate plants and plant products (Cheraghali et al., 2007; Iqbal et al., 2010). Natural forms of aflatoxin, including forms B_1, B_2, G_1, and G_2, are often found in feeds. Aflatoxin M_1 (**AFM_1**), the monohydroxylated derivate of aflatoxin B_1 (**AFB_1**), occurs in milk from dairy cows fed an AFB_1-contaminated diet and may be subsequently transferred into other dairy products (Battacone et al., 2005; Firmin et al., 2011). Both AFB_1 and AFM_1 are hepatotoxic and carcinogenic, and are classified as Group 1 human carcinogens by the International Agency for Research on Cancer (**IARC**) of the World Health Organization (IARC, 2002). Some countries and agencies pay close attention to aflatoxin contamination in feed and milk and have established legal limits for maximum residue levels (**MRL**) for AFB_1 in feeds for lactating cows and for AFM_1 in milk and dairy products. In the United States, the Food and Drug Administration (FDA) has set the MRL for total aflatoxins (AFB_1 + AFB_2 + AFG_1 + AFG_2) in lactating cow feeds and raw milk to be 20 μg/kg and 500 ng/kg, respectively (FDA, 2000). The MRL for AFB_1 in dairy cattle feeds and MRL for AFM_1 in raw milk set by the

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European Commission were 5 μg/kg (EC, 2001) and 50 ng/kg, respectively (EC, 2002); and in China, the MRL of AFB_1 in feed was 10 μg/kg (China General Administration of Quality Supervision, Inspection and Quarantine, 2001) and that of AFM_1 in raw milk was 500 ng/kg (China Ministry of Health, 2011). The MRL for AFM_1 set by China and the United States is 10 times the level set by the European Commission.

In practice, feedstuffs such as corn, cottonseed, and corn silage may be contaminated by aflatoxins when plants grow in the field and are stored under inadequate storage conditions (González Pereyra et al., 2008; Richard et al., 2009), potentially resulting in AFM_1 contamination of milk when the feed is consumed by lactating dairy cows (Unusan, 2006; Xiong et al., 2013). The AFM_1 is then introduced into the human body through consumption of milk or milk products (Galvano et al., 1998), leading to adverse health problems. Aflatoxins in diets of cattle have been reported to impair liver function (Garrett et al., 1968), reduce milk yield and quality, impair immune response, and increase susceptibility to infectious diseases (Masoero et al., 2007; Queiroz et al., 2012). Compared with fattening cattle, dairy cows are considered more sensitive to aflatoxins (Applebaum et al., 1982).

To lessen the transfer of aflatoxins from feed to milk, researchers have tried various methods including physical, chemical, or biological treatments (Bhat et al., 2010; Xiong et al., 2012). Among these methods, utilization of adsorbent made from bentonite and esterified glucomannan is regarded as a promising and practical way to reduce aflatoxin absorption in the gastrointestinal tract (Diaz et al., 2004; Kabak et al., 2006). In most instances, organic or inorganic adsorbent products have been used to investigate the effects of adsorbent on the transfer of dietary AFB_1 to milk AFM_1. In the study of Stroud (2006), addition of hydrated sodium calcium aluminosilicates at 0.5% of diet resulted in a 40% reduction in milk AFM_1 in dairy cows ingesting 170 μg of AFB_1/kg of DM. Adding a 0.56% modified yeast cell culture preparation to the diet caused a 4% reduction in milk AFM_1 concentration in dairy cows receiving 112 μg of AFB_1/kg of DM (Kutz et al., 2009). Queiroz et al. (2012) found a 17% reduction in milk AFM_1 in response to adding hydrated sodium calcium aluminosilicates at 1% of the diet.

Even though adsorbents have been widely used in diets of lactating dairy cows in China, no study has determines the transfer of dietary AFB_1 to milk AFM_1 and the effect of including Solis Mos (Novus International Inc., St. Charles, MO) in the diet of dairy cows in China. Furthermore, information is limited on the effect of adsorbent on oxidative stress, ruminal fermentation, and milk AFM_1 secretion in dairy cows. The objectives of this study were to investigate the transfer of aflatoxin from feed to milk and to evaluate the effect of Solis Mos on the transfer of aflatoxin to milk, on indicators of oxidative stress, and on ruminal fermentation in mid-lactation dairy cows receiving 1 of 2 doses of AFB_1.

MATERIALS AND METHODS

Preparation of Aflatoxins and Adsorbent

The AFB_1 used for the experiment was produced by cultivating toxigenic *Aspergillus flavus* (No. 3.4409) obtained from China General Microbiological Culture Collection Center (Beijing, China). The ingredients used as a carrier for the aflatoxin were as follows: 75% long-shaped rice, 10% sucrose, 10% soybean meal, and 5% wheat bran. All ingredients were ground to pass through 0.9-mm mesh sieve and mixed completely. The grain carrier was adjusted to 20% moisture and placed in a 50- × 30-mm sterilized square plate with a gas-permeable cap and sterilized by autoclaving (121°C, 20 min). The carrier was inoculated with *A. flavus* and incubated at 25°C for 3 wk. Then, the inoculated carrier was autoclaved (121°C, 20 min) to stop fungal growth and dried at 75°C for 24 h. Culture materials were analyzed by the HPLC method (China Ministry of Health, 2006), as described below. The concentrations of AFB_1 and AFB_2 were 28.8 and 0.3 mg/kg, respectively, with AFG_1 and AFG_2 being undetectable.

Solis Mos, a proprietary additive from Novus International Inc., was a mixture of sodium montmorillonite with live yeast, yeast culture, mannan oligosaccharide, and vitamin E.

Animals, Experimental Design, and Diets

The experiment was conducted at Hangjiang Dairy Farm (Hangzhou, China) from December 15, 2012, to January 21, 2013, and was in accordance with the guidelines for animal research at Zhejiang University. Three groups of 8 multiparous Holstein cows in late lactation (DIM = 271 ± 29 d, milk yield = 21.6 ± 3.1 kg/d) were assigned to 1 of 3 trials in a 2 × 2 crossover design. Cows in experiment 1 received no aflatoxin, cows in experiment 2 received 20 μg of AFB_1/kg of diet DM, and cows in experiment 3 received 40 μg of AFB1/kg of diet DM. Cows in each experiment were assigned to 1 of 2 dietary treatments: control (**CON**) or addition of Solis Mos at 0.25% of the diet (DM basis; **SM**). Each experiment consisted of 2 consecutive periods with an adaptation for 4 d (d 1 to 4), when all cows were fed the basal diet without addition of AFB_1 or SM; AFB_1 challenge for 7 d (d 5 to 11); and clearing

for 5 d (d 12 to 16) each period. In the second period, cows remained on the same AFB_1 level but received the other treatment. Similar durations of adaptation, dosing, and clearance were used in the studies by Masoero et al. (2007) and Queiroz et al. (2012). The health condition of the cows was monitored daily during the entire period.

Diets were formulated according to nutrient requirements (China Ministry of Agriculture, 2004) for late-lactation Holstein cows weighing 600 kg and producing 25 kg/d of milk. Ingredients in the basal diet are shown in Table 1. The daily doses of AFB_1 and adsorbent were divided into 3 aliquots, and each aliquot was mixed with 100 g of corn meal to encourage consumption. The AFB_1 and SM mixture was fed to cows in a separate container during d 5 to 11 of each period. The TMR was not offered to cows until the mixture was fully consumed to ensure the cows ate all of the toxin dose and adsorbent. Cows receiving the CON treatment in experiment 1 were fed 100 g of corn meal followed by the TMR at each feeding. Cows were housed in a tie-stall barn bedded with sawdust and fed their daily TMR at 0700, 1300, and 1900 h with free access to water. Cows were milked 3 times per day at 0630, 1230, and 1830 h. The DMI of each individual cow and individual milk yields were recorded on d 1, 2, and 10 to 14 of each period.

Sample Collection and Analysis

The samples of TMR and milk were collected on d 1, 2, and 10 to 14 of each period. The TMR samples were dried at 65°C for 48 h in a forced-air oven, and then ground to pass through a 0.9-mm mesh sieve using a high-speed grinder (HK180, Xuliang Mechanical Equipment Co. Ltd., Guangdong, China) and stored at −20°C until analysis of aflatoxin and chemical composition. The DM (method no. 930.5), CP (method no. 984.13), ash (method no. 942.05), and ADF (method no. 973.18) were determined using AOAC International (2000), and NDF was analyzed using methods outlined in Van Soest et al. (1991). Ingredients and chemical composition of the basal diet are shown in Table 1.

Milk samples (approximately 300 mL) collected from each of the 3 daily milkings were mixed completely in a ratio according to the milk yield from each milking, and then subsequently divided into 3 equal aliquots (100 mL each). A bronopol preservative (Broad Spectrum Microtabs II D&F Control System Inc., Dublin, CA) was added to milk samples before storage. A group of milk samples was immediately sent to the Shanghai Dairy Herd Improvement testing center for analysis of milk fat, protein, and SCC using a Combi Foss FT+ instrument (Foss Electric, Hillerød, Denmark). Another group of milk samples was stored at −20°C until analysis of milk AFM_1.

Blood samples (10 mL) were collected from the coccygeal vein into heparinized vacuum tubes before the morning feeding on d 1, 11 and 14 each period, respectively, and then centrifuged at 3,000 × *g* for 10 min at 4°C to obtain plasma, which was frozen at −20°C until later analysis. Plasma samples were analyzed using an Auto-Analyzer 7020 (Hitachi High-Technologies Corp., Tokyo, Japan) with colorimetric commercial kits (DiaSys Diagnostics Systems GmbH, Holzheim, Germany) for glutamic oxalacetic transaminase (**GOT**, No. 14126070202), glutamic pyruvic transaminase (**GPT**, No. 14127070201), alkaline phosphatase (**ALP**, No. 14104170202), γ-glutamyl transferase (**GGT**, No. 14128070202), and total bilirubin (**TBIL**, No. 14108170201). The plasma levels of GOT, GPT, ALP, GGT, and TBIL were analyzed as described by Thomas (1998). One set of plasma samples was used to determine the concentrations of IgG (No. CK-E92027), IgM (No. CK-E92029), and IgA (No. CK-E92028) with bovine IgG, IgM, and IgA ELISA kits (Shanghai Yanhui BioTech Company, Shanghai, China). The plasma concentrations of IgG, IgM, and IgA were analyzed using an immunoturbidimetry assay (Thomas, 1998). Another set of plasma samples was sent to Nanjing Jiancheng Bioengineering Institute (Nanjing, Jiangsu, China) for determination of the level of total antioxidant capacity (**TAOC**, No. A015), superoxide dismutase (**SOD**, No.

Table 1. Ingredients and chemical composition of the basal diet

Item	Amount
Ingredient composition (% of DM)	
Corn silage	17.9
Chinese wild rye	29.1
Dried beet pulp	4.2
Carrot	5.2
Distillers grain	8.7
Corn	19.6
Soybean meal (42.5% CP)	4.5
Wheat bran	4.3
Cottonseed meal	4.0
Barley	1.2
Sodium chloride	0.45
Calcium hydrogen phosphate	0.35
Premix[1]	0.50
Chemical composition	
DM (%)	44.9
CP (% of DM)	15.8
Ash (% of DM)	8.8
NDF (% of DM)	38.3
ADF (% of DM)	21.3
Ca (g/kg of DM)	1.0
P (g/kg of DM)	0.6
Aflatoxins B_1, B_2, G_1, and G_2 (μg/kg of DM)	ND[2]

[1]Formulated to provide (per kg of DM): 912,000 IU of vitamin A, 122,000 IU of vitamin D_3, 2,000 IU of vitamin E, 877 mg of Cu, 482 mg of Fe, 368 mg of Mn, and 1,754 mg of Zn.

[2]Not detected.

A001-1), glutathione peroxidase (**GSH-Px**, No. A005), and malondialdehyde (**MDA**, No. A003-1). Activities of SOD and GSH-Px and content of MDA were measured by reacting the samples with hydroxylamine, followed by a catalytic reaction of H_2O_2 and GSH and a subsequent reaction with thiobarbituric acid (Zhang et al., 2006). The TAOC was measured using a ferric-reducing/antioxidant power assay (Benzie and Stain, 1996).

Rumen fluid was collected by oral stomach tube (Shen et al., 2012) 2 h after the morning feeding on d 1 and 11 of each period. To reduce contamination of rumen fluid by saliva, the first 150 mL of rumen fluid was discarded, and the oral stomach tube was washed using running water after sampling each cow. The pH value of rumen fluid was immediately determined using a Starter 300 pH meter (Ohaus Corp., Parsippany, NJ). Rumen fluid from each cow was then placed into a 2-mL centrifuge tube and stored at −72°C until analysis of VFA. The concentrations of acetate, butyrate, and propionate were determined as described by Hu et al. (2005) using a gas chromatograph (GC-2010, Shimadzu Corporation, Kyoto, Japan) equipped with a flame-ionization detector and a capillary column (HP-Innowax, 1909N-133, Agilent Technologies, Santa Clara, CA).

Determination of Aflatoxins in Diets and Milk

Aflatoxins B_1, B_2, G_1, and G_2 in all feed samples were determined using the HPLC method as described by the China Ministry of Health (2006). Briefly, aflatoxins in feed were extracted using a mixture of acetonitrile:water (84:16, vol/vol). Two milliliters of purified eluate was evaporated to approximate dryness under N at 60°C and mixed with hexane and trifluoroacetic acid. The mixture was derived at 40°C for 15 min and dissolved by acetonitrile:water (15:85, vol/vol). The solution was centrifuged, and the supernatant was used for the detection of aflatoxins with a Hewlett-Packard 1100 HPLC system (Hewlett-Packard, Palo Alto, CA) connected to a Zorbax SB reversed-phase C_{18} (150 × 4.6 mm i.d., 5 μm) column (Agilent Technologies, Palo Alto, CA). The minimum level of detection was set at 0.3 μg/kg for aflatoxins B_1 and G_1, and 0.2 μg/kg for aflatoxins B_2 and G_2.

The AFM concentration of all the collected milk samples was measured using liquid chromatography-tandem MS (LC-MS/MS) as described by Xiong et al. (2013). The minimum level of detection was set at 10 ng/L.

Calculations

Milk AFM1 transfer variables were calculated as follows:

$$\text{Secretion (ng/d)} = \text{concentration of AFM}_1 \text{ in milk (ng/L)} \times \text{milk yield (kg/d)}, \text{ and}$$

$$\text{Transfer (\%)} = \text{excretion of AFM}_1 \text{ (ng/d)} / \text{AFB}_1 \text{ consumption (ng/d)} \times 100\%.$$

The SCC were divided by 1,000 and converted to the natural logarithm before analysis.

Statistical Procedures

The experiment was arranged as a 2 × 2 crossover design, and the 2 periods were consecutive. In the second period, cows from the 2 treatments in each experiment exchanged diets without changing AFB_1 level. The interval when no AFB_1 was administered (between the last AFB_1 administration in period 1 and the beginning AFB_1 administration in period 2) was 9 d. Data on DMI, milk yield, milk composition, milk AFM_1 transfer variables, plasma biochemical parameters, and rumen fluid VFA data were analyzed by ANOVA using the MIXED procedure of SAS (version 9.2, SAS Institute Inc., Cary, NC). The statistical model includes the fixed effects of period, treatment, and treatment × period, and cow within square as the random effect. The day of collection was included in the model as a repeated measure (compound symmetry covariance structure). The data were checked for normality by Levene's test. Given the non-normal distribution of some data on plasma and rumen fluid, the differences among treatments were compared by Mann-Whitney U test.

The data of DMI, milk yield, milk composition, plasma biochemical parameters, and ruminal VFA proportion obtained from the first day of the adaptation period were added to the model as a covariate in the statistical analysis concerned. Tukey's adjustment test was used to determine significant differences between least squares means. All statements of statistical significance were based on a probability of $P < 0.05$. Trends were discussed at a statistical significance of $0.05 < P \leq 0.10$.

RESULTS AND DISCUSSION

Feed Intake, Milk Yield, and Milk Composition

The inclusion of SM in the diets without or with AFB_1 contamination had no effect ($P > 0.05$, Table 2) on DMI, milk yield, or milk composition. Similarly, Kutz et al. (2009) showed no difference of DMI, milk yield, or milk composition in dairy cows fed a combination of AFB_1 (112.2 μg/kg of DM) and adsorbent (clay

or yeast cell culture as the main ingredients; 125 g/d). Feeding of an adsorbent (yeast cell wall extract as the main ingredient; 2 g/kg of feed) alone or a mixture of adsorbent and AFB_1 (60 μg/kg) did not affect DMI, milk yield, or milk composition in dairy ewes (Firmin et al., 2011). In addition, Queiroz et al. (2012) found that the addition of different doses of adsorbent (clay as the main ingredient; 0.2 or 1% of DM) in an AFB_1-contaminated diet (75 μg/kg of AFB_1) did not affect DMI, milk yield, or 3.5% FCM. Consistent with results from previous studies (Kutz et al., 2009; Firmin et al., 2011; Queiroz et al., 2012), the results in the current study demonstrated that addition of AFB_1 with adsorbent (0.25% of diet DM) did not influence lactation performance of dairy cows regardless of the level of AFB_1 inclusion (0, 20, or 40 μg/kg).

Transfer of AFB_1 from Feed to AFM_1 in Milk

Patterns of clearance in milk AFM_1 after 7 d of AFB_1 administration are shown in Figure 1. Milk AFM_1 concentrations in the treatment without AFB_1 (Figure 1a) were near 0 ng/L from d 11 to 14, as expected with the level of dietary AFB_1 being below the detection limits; however, an average level of milk AFM_1 of 3 ng/L was detected in cows fed diets with no supplemental AFB_1 (Table 2). The background AFM_1 in the milk might be related to sporadic aflatoxin contamination of dietary ingredients (corn, cottonseed meal, and corn silage) that were stored at 17.1°C and 71% relative humidity during the study. Previous researchers have demonstrated that feeds stored in a wide range of temperatures (13 to 42°C) and humidity (around 75% of relative humidity) easily produce AFB_1 that are distributed unevenly in the feed (Shotwell et al., 1975; Mostrom and Jacobsen, 2011; Battacone et al., 2012).

The addition of SM to the 20 μg/kg AFB_1-contaminated diet resulted in a 16.0% reduction ($P < 0.05$) in milk AFM_1 concentration, an 18.3% reduction ($P < 0.05$) in milk AFM_1 secretion, and a 17.9% reduction ($P < 0.05$) in transfer (Figure 1b; Table 2) on the final day of dosing AFB_1 (d 11), when we would have expected milk AFM_1 to be maintaining a stable concentration (Masoero et al., 2007). Concentrations of AFM_1 in milk were similar between the 2 treatments after termination of AFB_1 and SM administration (d 12 to 14).

We detected only a numerical reduction ($P > 0.10$) in milk AFM_1 level (2%), AFM_1 output (4.0%), and transfer (3.4%) with the addition of SM to the 40 μg/kg AFB_1-contaminated diet (Figure 1c; Table 2). The differences in adding SM on AFM_1 binding is attributable to the low dose of SM and the fact that clay adsorbents bind AFB_1 in a dose-dependent manner (Sarr, 1995). Queiroz et al. (2012) reported that a clay adsorbent

Table 2. Least squares means of DMI, milk production, milk aflatoxin M_1 (AFM_1), and transfer of aflatoxins from diet to milk in dairy cows fed control or a diet containing Solis Mos in 3 experiments with different dietary aflatoxin B_1 (AFB_1) levels (0, 20, and 40 μg/kg of DM)[1]

	0 μg/kg				20 μg/kg				40 μg/kg			
Item	CON	SM	SEM	*P*-value	CON	SM	SEM	*P*-value	CON	SM	SEM	*P*-value
DMI (kg/d)	17.3	17.1	0.95	0.91	17.0	17.6	0.38	0.14	17.4	17.2	0.60	0.76
Milk(kg/d)	21.3	22.1	0.52	0.32	21.3	21.3	0.54	0.95	22.4	22.6	0.61	0.85
3.5% FCM (kg/d)	25.6	27.0	0.96	0.36	25.2	26.0	0.68	0.28	27.1	27.3	1.52	0.92
Milk composition (%)												
Protein	3.08	3.05	0.108	0.84	3.11	3.08	0.044	0.69	3.07	3.08	0.087	0.95
Fat	3.71	4.01	0.139	0.20	3.85	3.71	0.183	0.63	3.73	3.86	0.236	0.71
Lactose	4.87	4.88	0.043	0.93	4.87	4.90	0.032	0.25	4.91	4.91	0.047	0.91
SCC (log cells/μL)	4.88	5.42	0.470	0.45	5.31	5.68	0.258	0.36	5.00	5.01	0.568	0.98
AFM_1 in milk[2]												
ng/L	3.1	ND[3]	1.81	0.32	105	88	4.5	0.04	208	204	19.8	0.88
ng/d	59.4	ND	35.17	0.34	2,249	1,837	88.8	0.03	4,738	4,549	496.5	0.79
Transfer[4] (%)	—	—	—	—	0.56	0.46	0.022	0.03	0.59	0.57e	0.06	0.79

[1]CON = control; 0 μg of AFB_1/kg of diet DM; SM = Solis Mos (Novus International Inc., St. Charles, MO), an adsorbent containing sodium montmorillonite with live yeast, yeast culture, mannan oligosaccharide, and vitamin E.

[2]Amount of AFM_1 excreted in milk.

[3]ND = not detected. For statistical analysis, the not-detected value was set to be 5 ng/kg (mean of 0 and 10 ng/kg, detection limit value).

[4]Percentage of AFM_1 excreted in milk to the ingested AFB_1.

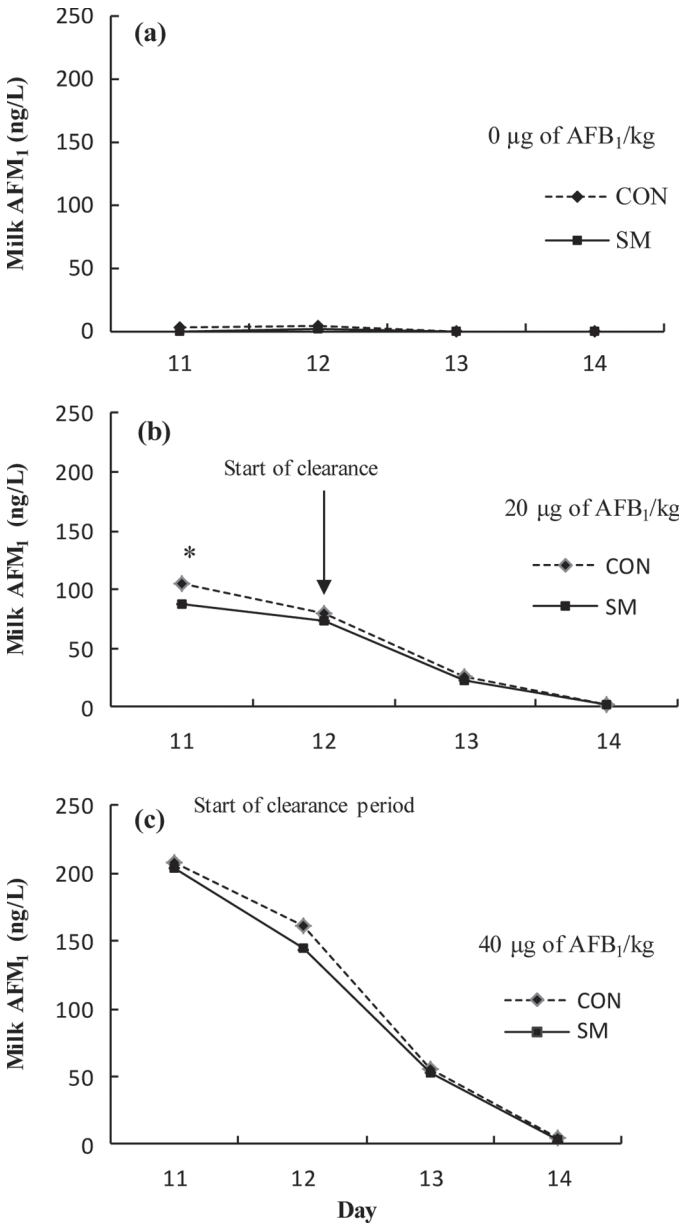

Figure 1. Trends of aflatoxin M_1 (AFM_1) concentration in the milk of cows fed (a) 0, (b) 20, or (c) 40 μg of aflatoxin B_1 (AFB_1)/kg of DM without (control, CON) or with Solis Mos (SM, an adsorbent containing sodium montmorillonite with live yeast, yeast culture, mannan oligosaccharide, and vitamin E; Novus International Inc., St. Charles, MO) after the last day of AFB_1 administration. Dietary AFB_1 and milk AFM_1 on d 4, the last day of the adaption period, were undetected and 3 ng/L on average, respectively. Each data point represents the mean ± SD of 8 dairy cows; $^*P < 0.05$ between treatments.

added to a TMR at 0.2% of DM did not affect the concentration of AFM_1 in the milk of dairy cows fed 75 μg of AFB_1/kg of feed, but significantly decreased the milk AFM_1 concentration when fed at a level of 1% of diet DM. Compared with Solis studied by Kutz et al. (2009), the SM in this study was less efficacious in reducing milk AFM_1 concentration, probably due to the dose (0.56 vs. 0.25%), and potentially due to the different ingredients in their diets and in the composition of the Solis and SM product. The Solis used by Kutz et al. (2009) was a source of hydrated sodium calcium aluminosilicates, whereas SM was a mixture of Solis, yeast culture, yeast extract, and vitamin E. Firmin et al. (2011) and Battacone et al. (2009) confirmed that modified yeast cell wall and dried yeast culture added to the feed at a level of 2 g/kg or 12 g/d did not affect the concentration of AFM_1 in milk of dairy ewes fed 60 μg of AFB_1/kg of feed, or 1.13 to 5.03 μg of AFB_1/kg of feed. In contrast, Diaz et al. (2004) found that the addition of a yeast cell extract (MTB-100) at 0.05% of a diet containing 55 μg of AFB_1/kg reduced milk AFM_1 concentration in dairy cows by 59%. However, MTB-100 was not effective at reducing milk AFM_1 concentrations when added at 0.56% to a dairy cow diet containing 112 μg of AFB_1/kg (Kutz et al., 2009). The difference in effect of yeast cell wall on milk AFM_1 may be related to the species of the experimental animals and the composition of the adsorbent. Although 40 μg/kg AFB_1 used in this study was twice the US legal limit for aflatoxins and 4 times the Chinese legal limit, levels higher than the limit have been detected in China; for instance, 134 μg/kg of AFB_1 in corn (Yang et al., 2012). Therefore, further work is needed to study the effects of types and dosage of adsorbent on milk AFM_1 clearance and healthy condition. The AFM_1 in milk quickly decreased to undetectable levels 3 d after AFB_1 administration was stopped, regardless of whether the level of dietary AFB_1 was 20 or 40 μg/kg (Figure 1). Similarly, Masoero et al. (2007) and Queiroz et al. (2012) reported that milk AFM_1 content in all milk samples was reduced to undetectable levels after 72 h of the AFB_1 withdrawal period. Frobish et al. (1986) showed that AFM_1 in milk of dairy cows decreased to normal levels within 3 to 4 d of the final AFB_1 administration. Moreover, Battacone et al. (2012) confirmed that AFM_1 in milk of goats was not detected 84 h after a single dose of AFB_1 ingestion. The current study further confirmed that milk AFM_1 was reduced to undetectable levels 3 d after the last AFB_1 administration, even when relatively high levels (40 μg/kg, twice the US legal limit) of AFB_1 was fed.

Plasma Parameters

Plasma parameters of dairy cows are shown in Table 3. Adding SM to the AFB_1-free diet decreased ($P < 0.01$) the concentration of MDA and increased ($P < 0.01$) the SOD:MDA ratio. Addition of SM in the AFB_1-contaminated diets (20 μg/kg of AFB_1) decreased ($P < 0.05$) the concentration of MDA, but led to the same increase ($P < 0.05$) in the SOD level and the SOD:MDA ratio. Moreover, inclusion of SM in the

AFB_1-contaminated diets (40 μg/kg AFB_1) tended to decrease ($P = 0.09$) the MDA level, but increased ($P < 0.05$) the concentration of SOD and thus the SOD:MDA ratio. In the present study, the levels of GSH-Px and TAOC did not differ between SM and CON treatments at all 3 levels of AFB_1 addition.

Superoxide dismutase plays an important role in the conversion of oxygen radicals to peroxides (Yu, 1994), whereas MDA is a lipid peroxidation product that is an indicator of oxidative stress (Armstrong and Browne, 1994). The lower plasma MDA and greater SOD in cows fed SM in the current study demonstrated that SM reduced oxidative stress in the cows. The reduction in indicators of oxidative stress may be related to the increased supply of vitamin E, yeast extract, and montmorillonite in the SM diet compared with CON. Verma and Nair (2001) confirmed that a vitamin E pretreatment could ameliorate aflatoxin-induced lipid peroxidation in the testis of mice. Alpsoy et al. (2009) also reported that vitamin E tended to lower MDA in human lymphocytes treated with AFB_1. In the work of Zhang et al. (2005), the addition of yeast extract to the diet had oxidation-reducing effects on broiler chicks. Other studies have documented that yeast walls have antioxidant properties (Pinheiro et al., 2002; Kogan et al., 2005). In the study of Shi et al. (2006), aflatoxin-contaminated diets decreased activities of serum SOD and increased levels of serum MDA in broiler chicks, whereas addition of montmorillonite increased activities of serum SOD and decreased levels of serum MDA. The montmorillonite in SM may have decreased gastrointestinal absorption of AFB_1 by binding AFB_1 and, therefore, alleviated oxidative stress.

Plasma GOT, GPT, GGT, and ALP have been proposed as indicators of depressed liver function (Miller et al., 1981), and no differences were observed in these indicators in the current study (Table 3). In addition, the plasma concentrations of antibodies, IgG, IgM, and IgA were within normal physiological ranges throughout the experimental period and were not changed by SM addition, suggesting that SM at the current dose did not affect liver function or immune condition within the cow's body. However, Queiroz et al. (2012) observed that the concentration of haptoglobin as an indicator of innate immune stress decreased when dairy cows were fed additional clay adsorbent as part of a diet with a high level of AFB_1 (75 μg/kg of AFB_1), which was a higher dose than used in the current study.

Consistent with the present study, Battacone et al. (2005) reported that ingestion of 12 g/d of adsorbent (dry yeast culture) resulted in no significant changes in concentrations of plasma ALP, GOT, or GPT of dairy sheep fed an AFB_1-contaminated diet (1.13, 2.30, and 5.03 μg of AFB_1/kg of feed). Fu et al. (2013) found no

Table 3. Least squares means of plasma parameters for dairy cows fed a control diet or a diet containing Solis Mos in 3 experiments with different dietary aflatoxin B_1 (AFB_1) levels (0, 20, and 40 μg/kg of DM)[1]

	0 μg/kg				20 μg/kg				40 μg/kg			
Item[2]	CON	SM	SEM	*P*-value	CON	SM	SEM	*P*-value	CON	SM	SEM	*P*-value
SOD (U/mL)	72.0	71.7	1.26	0.80	71.3	75.6	1.13	0.04	70.0	74.3	2.43	0.02
MDA (nmol/mL)	3.3	2.7	0.17	0.01	3.6	3.2	0.14	0.04	3.7	3.1	0.25	0.09
SOD:MDA	22.2	31.9	2.26	<0.01	20.2	27.2	1.29	<0.01	19.3	27.7	1.70	<0.01
GSH-Px (U/mL)	116.8	80.6	18.26	0.21	98.8	102.6	12.61	0.80	101.9	99.2	16.86	0.92
TAOC (U/mL)	2.6	3.2	0.43	0.29	2.6	3.1	0.30	0.30	2.6	3.2	0.36	0.20
GOT (U/L)	87.0	90.3	3.21	0.49	87.8	90.1	4.38	0.72	87.2	84.6	2.08	0.40
GPT (U/L)	39.3	39.8	1.22	0.75	36.7	37.8	1.21	0.55	33.8	33.7	0.98	0.89
GGT (U/L)	147.9	151.1	3.15	0.50	146.4	143.0	4.0	0.56	140.3	149.9	5.73	0.15
ALP (U/L)	76.6	77.5	4.08	0.87	73.7	76.1	3.69	0.65	73.4	74.7	2.24	0.69
TBIL (μmol/L)	2.7	2.7	0.08	0.91	2.7	2.8	0.09	0.42	2.8	2.8	0.05	0.90
IgG (mg/L)	79.0	71.0	4.21	0.21	77.7	78.8	5.90	0.91	76.9	77.7	6.24	0.93
IgM (mg/L)	28.9	30.3	1.87	0.20	31.8	33.9	1.04	0.14	29.8	28.9	1.76	0.73
IgA (mg/L)	57.4	55.4	3.99	0.72	57.4	57.6	2.22	0.95	53.2	53.0	1.79	0.94

[1]CON = control; 0 μg of AFB_1/kg of diet DM; SM = Solis Mos (Novus International Inc., St. Charles, MO), an adsorbent containing sodium montmorillonite with live yeast, yeast culture, mannan oligosaccharide, and vitamin E.

[2]SOD = superoxide dismutase; MDA = malondialdehyde; GSH-Px = glutathione peroxidase; TAOC = total anti-oxygen capability; GOT = glutamic oxalacetic; GPT = glutamic-pyruvic transamine; GGT = γ-glutamyltranspeptidase; ALP = alkaline phosphatase; TBIL = total bilirubin.

effects of dietary addition of 1% maifanite (a type of aluminosilicate clay) on liver function parameters and antibody levels of piglets fed an AFB_1-contaminated diet (372.8 μg/kg of AFB_1).

VFA in Rumen Fluid

The pH of the rumen fluid was not influenced by the addition of SM regardless of the AFB_1 level added in the diet. The inclusion of SM in the AFB_1-free diet increased total VFA concentration ($P = 0.04$), tended to decrease the molar proportion of propionate ($P = 0.05$), and increased the acetate:propionate ratio ($P = 0.09$) in rumen fluid (Table 4), but did not affect ($P > 0.1$) the molar proportions of acetate or butyrate. Addition of SM to the AFB_1-contaminated diet (20 μg/kg of AFB_1) increased total VFA concentration ($P = 0.04$), tended to increase the acetate:propionate ratio ($P = 0.09$), and decreased the propionate proportion ($P = 0.09$). In contrast, adding SM to the AFB_1-contaminated diet (40 μg/kg of AFB_1) did not elicit changes in VFA fermentation profiles in the rumen fluid.

The effects of SM on ruminal VFA concentration and pattern may be related to the yeast culture in the SM product. Mohamed et al. (2009) observed that addition of a yeast culture at 5 g/kg of DM improved total VFA concentration in calves' rumen fluid, and Lascano and Heinrichs (2009) reported that addition of yeast culture to the diet of dairy heifers at 1 g/kg on an as-fed basis increased the total VFA concentration in rumen fluid. These previous results on rumen VFA concentrations are similar to those of the current study when the dietary AFB_1 was at 0 or 20 μg/kg. The tendency for a decreased propionate proportion potentially indicated a decrease in the degradation of NSC, because propionate production is mainly related to fermentation of NSC by amylolytic bacteria (Enjalbert et al., 1999). In an in vitro study, Mao et al. (2013) found a slight but significant increase in propionate proportion by addition of yeast culture, whereas Lascano and Heinrichs (2009) did not observe the effect of yeast culture on mean propionate proportion in Holstein heifers. These differences may be attributed to the variation in dietary ingredients, nutrient composition, or dosage of yeast culture. Moreover, addition of SM did not induce changes in total VFA concentration in dairy cows fed 40 μg/kg of AFB_1-contaminated diet, probably due to the level of SM fed and the lack of additional binding of AFB_1 by the SM.

Table 4. Least squares means of VFA proportions in rumen fluid of dairy cows fed a control diet or a diet containing Solis Mos in 3 experiments with different dietary aflatoxin B_1 (AFB_1) levels (0, 20, and 40 μg/kg of DM)[1]

	0 μg/kg				20 μg/kg				40 μg/kg			
Item	CON	SM	SEM	*P*-value	CON	SM	SEM	*P*-value	CON	SM	SEM	*P*-value
pH	6.37	6.38	0.090	0.92	6.51	6.42	0.070	0.35	6.61	6.50	0.053	0.20
Total VFA (m*M*)	94.2	99.6	1.54	0.04	93.4	99.8	1.96	0.04	92.8	98.8	2.70	0.15
Molar proportion (mol/100 mol)												
Acetate	71.6	71.0	0.47	0.40	70.1	71.1	0.47	0.15	70.2	70.3	0.67	0.86
Propionate	19.2	18.1	0.33	0.05	19.5	19.0	0.19	0.09	19.7	19.5	0.45	0.59
Butyrate	9.3	10.9	0.64	0.10	10.5	10.0	0.35	0.33	10.1	10.2	0.59	0.92
Acetate:Propionate	3.8	3.9	0.07	0.09	3.6	3.8	0.06	0.09	3.6	3.6	0.10	0.44

[1]CON = control (0 μg of AFB_1/kg of diet DM); SM = Solis Mos (Novus International Inc., St. Charles, MO), an adsorbent containing sodium montmorillonite with live yeast, yeast culture, mannan oligosaccharide, and vitamin E.

CONCLUSIONS

Adding SM at 0.25% of the diet to AFB_1-free or AFB_1-contaminated diets had no effect on lactation

performance but significantly decreased plasma concentration of MDA and increased that of SOD. The addition of SM to the AFB_1-free diet increased total ruminal VFA concentration and reduced the proportion of propionate. Adding SM to the diet contaminated with 20 μg/kg of AFB_1 decreased the AFM_1 concentration and the transfer of aflatoxin to milk, and increased the total VFA concentration. However, adding SM to the diet contaminated with 40 μg/kg of AFB_1 did not reduce AFM_1 concentrations in milk or alter VFA concentrations. The addition of adsorbent decreased milk AFM_1 concentration, improved antioxidative status, and altered rumen fermentation in dairy cows fed diets with a lower level of AFB_1 but no effect was detected when the absorbent was added to a diet containing a higher level of AFB_1. Thus, a higher dosage of adsorbent may be needed to reduce milk AFM_1 levels in cow consuming diets with higher levels of AFB_1.

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