Dietary Hydrated Sodium Calcium Aluminosilicate Reduction of Aflatoxin M<sub>1</sub> Residue in Dairy Goat Milk and Effects on Milk Production and Components<sup>1,2</sup>

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**ABSTRACT:** Lactating dairy goats were exposed to aflatoxin (100 and 200 ppb) and hydrated sodium calcium aluminosilicate at 1, 2, and 4% in two separate experiments. Naturally occurring low levels of aflatoxin M<sub>1</sub> (.009 ppb) were found in the milk of the control diet, whereas there were no detectable levels of aflatoxin M<sub>1</sub> in the milk of diets containing hydrated sodium calcium aluminosilicate in both experiments. In Exp. 1, no treatment-related differences in clinical behavior or significant difference in the feed intake, milk production, or milk component analyses were observed with 200 ppb of aflatoxin and 4% hydrated sodium calcium aluminosilicate. However, 4% hydrated sodium calcium aluminosilicate was responsible for an 86.9% reduction of aflatoxin M<sub>1</sub> residue in the milk of dairy goats. In Exp. 2, the combination of 1% hydrated sodium calcium aluminosilicate and aflatoxin at 100 ppb resulted in an overall reduction of aflatoxin M<sub>1</sub> residue by 51.9%, which represented a mean change of aflatoxin M<sub>1</sub> from .553 to .266 ppb of aflatoxin M<sub>1</sub> in the milk. The diet that contained 2% hydrated sodium calcium aluminosilicate and 100 ppb of aflatoxin further reduced aflatoxin residue by a mean change from .553 to .098 of ppb aflatoxin M<sub>1</sub>, which represents an 82.2% reduction of aflatoxin M<sub>1</sub> residue in the milk. Analysis of the data by time indicated that there were no statistical differences between days of sampling. Information regarding the ability of hydrated sodium calcium aluminosilicate to prevent or reduce the level of aflatoxin M<sub>1</sub> residues in milk is critically needed. This finding has important implications, because milk is ultimately consumed by humans and animals, and the reduction of aflatoxin contamination in the milk could have an important impact on their health.

**Key Words:** Aluminosilicate, Aflatoxins, Goats, Milk


**Introduction**

Mycotoxins comprise a structurally diverse family of fungal-elaborated substances, many of which have been strongly considered as chemical precursors of toxicity in humans and animals. Undoubtedly, the most thoroughly studied and best understood of the mycotoxins are the aflatoxins. The aflatoxins have invoked much concern because of their toxicity and potent carcinogenicity (Loury and Hsieh, 1984).

Aflatoxin M<sub>1</sub>, the major metabolite of aflatoxin B<sub>1</sub> in goat's milk (Goto and Hsieh, 1985), was excreted from less than 1 to 3% of the ingested dose of aflatoxin B<sub>1</sub> (Polan et al., 1974; Applebaum et al., 1982). In a recent series of studies, hydrated sodium calcium aluminosilicate was evaluated for the detoxification of aflatoxin-contaminated feedstuffs (Phillips et al., 1990). Dietary addition of hydrated sodium calcium aluminosilicate has been shown to significantly decrease the levels of aflatoxin M<sub>1</sub> in the milk of lactating dairy cows (Harvey et al., 1991b). These findings suggest that adsorption of aflatoxin with materials such as hydrated sodium calcium aluminosilicate may provide a safe and field-practical method for the prevention of aflatoxicosis in animals and reduction of aflatoxin in food of animal origin. Consequently, this study was designed to evaluate the

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effects of hydrated sodium calcium aluminosilicate on milk production and milk composition in goats and to determine the efficacy for reducing aflatoxin M₁ residues in goat's milk.

**Materials and Methods**

Two experiments were conducted to evaluate the potential of hydrated sodium calcium aluminosilicate to reduce aflatoxin M₁ residue in lactating goat's milk. These experiments were conducted at the International Dairy Goat Research Center, Prairie View A&M University. The first approach was to evaluate the effects of dietary hydrated sodium calcium aluminosilicate (4%) on milk production and composition, and on the reduction of aflatoxin M₁ in the milk of dairy goats consuming aflatoxin-contaminated diets. The second study was conducted to determine the potential of dietary hydrated sodium calcium aluminosilicate (1 and 2%) to reduce aflatoxin M₁ residue in the milk of dairy goats consuming aflatoxin-contaminated diets. All animals were cared for and handled following NIH guidelines for the use and care of laboratory animals (NIH, 1985), and the experimentation was approved by the institutions' animal care and use committees.

**Chemicals.** Hydrated sodium calcium aluminosilicate (Novasil™) was provided by Engelhard (Beachwood, OH).

**Preparation and Administration of Aflatoxin.** Aflatoxin used was produced through the fermentation of rice by *Aspergillus parasiticus* NRRL 2999 as was previously described by Shotwell et al. (1966) and further modified by West et al. (1973). The fermented rice was autoclaved, ground to powder, and the aflatoxin in the rice powder was analyzed by HPLC and quantified spectrophotometrically (Nabney and Nesbitt, 1965; Wiseman et al., 1967) and was found to contain 79% aflatoxin B₁, 16% aflatoxin G₁, 4% aflatoxin B₂, and 1% aflatoxin G₂ based on total concentration of aflatoxin in the rice powder. The aflatoxin was incorporated into the diet by first mixing the appropriate quantities of rice with a 1-kg portion of the diet. The 1 kg of diet containing the toxin was then mixed with the remainder of the basal diet, with or without hydrated sodium calcium aluminosilicate.

**Goats.** In Exp. 1, 32 lactating Alpine goats were selected from the herd at the International Dairy Goat Research Center, based on comparable milk production during the previous week. These goats were randomly assigned to one of four groups, each with eight goats. These goats were fed complete diets balanced to meet the nutrient requirements of early lactating goats. The goats were fed in Kalan feeders and housed in a covered, open-air barn. Goats were given ad libitum access to water. In Exp. 2, 18 lactating Alpine goats from the International Dairy Goat Research Center were used. These goats were randomly assigned to one of six groups, each with three goats. Animal husbandry and selection based on comparable milk production for Exp. 2 were as described for Exp. 1.

**Experimental Design (Exp. 1).** In the first experiment, there were four dietary treatment groups: 1) control diet containing no added aflatoxin or hydrated sodium calcium aluminosilicate; 2) diet containing 4% hydrated sodium calcium aluminosilicate; 3) diet containing 200 µg of aflatoxin/kg of feed; and 4) diet containing 4% hydrated sodium calcium aluminosilicate and 200 µg of aflatoxin/kg of feed. All goats were provided equal portions (based on herd mean dairy milk production) of feed daily. Individual daily feed intake and milk production were recorded. Goats were observed and milked twice daily. Morning milk samples (300 mL) were collected from each goat on d 2, 4, 6, and 8 for determination of milk composition and analysis for aflatoxin M₁ by HPLC (Dept. of Veterinary Anatomy and Public Health, Texas A&M University).

**Experiment Design (Exp. 2).** Six dietary treatment groups were in Exp. 2: 1) control diet containing no added aflatoxin or hydrated sodium calcium aluminosilicate; 2) diet containing 1% hydrated sodium calcium aluminosilicate; 3) diet containing 2% hydrated sodium calcium aluminosilicate; 4) diet containing 100 µg of aflatoxin/kg of feed; 5) diet containing 1% hydrated sodium calcium aluminosilicate and 100 µg of aflatoxin/kg of feed; and 6) diet containing 2% hydrated sodium calcium aluminosilicate and 100 µg of aflatoxin/kg of feed. The provision of the diet and the procedure for milk sample collection and analysis were carried out as in Exp. 1, except that milk samples were collected on d 2, 4, 6, 10, and 12.

**Preparation of Milk Samples for Analysis.** Milk samples were prepared for analysis by modification of an existing method by Takeda (1984). Aliquots of milk (30 mL) were removed and diluted to 60 mL with distilled water in a 60-mL disposable syringe. A C₁₈ cartridge (SEP-PAK C₁₈, Waters Associates, Milford, MA) was prewashed with 10 mL of acetoni-trile and 10 mL of distilled water, using a disposable 20-mL syringe. Next, the milk sample was loaded onto the cartridge and eluted at a flow rate of 10 mL/min. The eluate was discarded. The cartridge (which contained the bound aflatoxin M₁) was then washed with 10 mL of basic 10% acetonitrile (ammonium hydroxide:acetonitrile:water, 1:10:90, vol/vol/vol) followed by 10 mL of acidic 10% acetonitrile (acetic acid: acetonitrile:water, 1:10:90, vol/vol/vol) at a flow rate of 10 mL/min. The eluate was discarded. Five milliliters of acidic 30% acetonitrile (acetic acid: acetonitrile:water, 1:30:70, vol/vol/vol) were added to the cartridge, and the eluate was collected into a 20-mL test tube. Two milliliters of methylene chloride were added. The tube was vortexed and then cen-trifuged to break the emulsion. The methylene chlo-
ride layer was transferred to a clean tube, and the extraction procedure was repeated. The extracts were pooled and concentrated to < .5 mL under a gentle stream of nitrogen gas and transferred to a limited volume insert (Waters Associates). The extract was completely evaporated under nitrogen and then dissolved in 200 μL of the acidic 30% acetonitrile solution. A volume of 150 μL was injected and analyzed for aflatoxin MI by HPLC.

Extracted milk samples were analyzed by an HPLC system consisting of a Waters 6000A solvent delivery system and a WISP 710B sample processor for sample injections (Waters Associates). Samples were eluted isocratically on a radically compressed 10-μm octadecylsilane cartridge (Waters Associates) with a mobile phase of acetonitrile:methanol:water (15:15:70) at a flow rate of 2 mL/min. A prefilter was placed between the injector and the cartridge. The aflatoxin MI was detected fluorometrically (excitation wavelength, 365 nm; emission wavelength, 425 nm) with a fluorescence detector (model 420C, Waters Associates). The HPLC chromatograms were recorded on a Waters Data Module (Waters Associates) at a chart speed of 1.0 cm/min. The concentration of aflatoxin MI in milk samples was determined by peak area and comparison with samples containing known concentrations of aflatoxin MI.

Data Analysis. All data (n = 32 and 18 in Exp. 1 and 2, respectively) were analyzed by ANOVA (Ott, 1988) using the GLM procedures for factorial arrangement of treatments (SAS, 1985). Treatment means were ranked by Dunnett t-test for comparison of all treatments against the aflatoxin group (SAS, 1985). All statements of differences are based on significance at P < .05.

Results

Feed Intake and Milk Production. Exposure of dairy goats to aflatoxin (100 and 200 ppb) singly or in combination with hydrated sodium calcium aluminosilicate (1, 2, or 4%) in Exp. 1 and 2 did not result in any treatment-related differences in clinical behavior or significant differences in feed intake or milk production. In Exp. 1 the average daily feed intake ranged from 2.64 to 2.82 kg/d. The mean value for the morning milk production ranged from .64 to .73 kg. Similar averages were obtained for Exp. 2.

Proximate Analysis. Data were collected and reported in the present study on the chemical components of milk (total solids, percentage of milk fat, percentage of milk protein, and percentage of milk lactose) only for Exp. 1. The calculated treatment mean values for total solids ranged from 9.85 to 10.28%. The mean value for milk fat ranged from 1.85 to 2.35%, and milk protein was found to be 3.13 to 3.71%. The milk lactose mean range was 3.04 to 3.23%. There were no differences (P > .05) for milk components between the treatment groups.

Aflatoxin MI in Milk (Exp. 1). The effects of hydrated sodium calcium aluminosilicate (4%) on the average daily levels of aflatoxin MI in dairy goat's milk are presented in Table 1. Naturally occurring low concentrations of aflatoxin were found present in the control diet. The aflatoxin MI concentrations found in milk from the control goats ranged from .005 to .037 ppb. On d 6 of the first experiment, concentrations as high as 1.433 ppb of aflatoxin MI were detected in milk from the aflatoxin-alone treatment group. However, a statistically significant reduction of aflatoxin MI was observed when aflatoxin was combined with 4% hydrated sodium calcium aluminosilicate in the diet of dairy goats. The lowest concentration of aflatoxin MI (.163 ppb) was observed on d 4 of milk sampling. The overall effects of the treatment combining aflatoxin plus 4% hydrated sodium calcium aluminosilicate on aflatoxin MI residues in goat’s milk are presented in Figure 1. The graph illustrates a significant interaction between hydrated sodium calcium aluminosilicate at 4% and aflatoxin at 200 ppb, resulting in an 86.9% reduction of aflatoxin MI residue in the milk of dairy goats.

Aflatoxin MI in Milk (Exp. 2). The results of daily residual concentrations of aflatoxin MI that were observed in the second experiment are presented in
lower ($P < .05$) than in the milk from goats fed the diet with aflatoxin alone. In contrast, when aflatoxin was combined with 1% hydrated sodium calcium aluminosilicate, only milk residues from d 4 and 6 were lower ($P < .05$) than milk residues from goats fed the aflatoxin-alone diet.

The overall treatment mean values of aflatoxin $M_1$ residue for the second experiment are represented in Figure 2. The combination of 1% hydrated sodium calcium aluminosilicate and aflatoxin at 100 ppb resulted in an overall reduction of aflatoxin $M_1$ residue by 51.99%, a mean change from .553 ppb to .286 ppb. Further reduction (82.2%) of aflatoxin $M_1$ residue was observed with the diet that contained 2% hydrated sodium calcium aluminosilicate and aflatoxin by a mean change from .553 ppb of aflatoxin $M_1$ to .098 ppb. Analysis of the data for time effects indicated that there were no differences ($P > .05$) between days of sampling.

**Discussion**

Aflatoxins are commonly found as toxic contaminants of human food in the tropics and have been detected in the milk of animals primarily as aflatoxin $M_1$ and $M_2$ (Maxwell et al., 1989). The acute toxicity and carcinogenicity of aflatoxin $M_1$, as well as its frequent occurrence in dairy products, have been well documented (Goto and Hsieh, 1985). Aflatoxin $M_1$ is just as toxic as aflatoxin $B_1$ (Pong and Wogan, 1971; Purchase, 1971) but not as carcinogenic or mutagenic (Hsieh, 1979). Depending on the dosage, aflatoxin $M_1$ can cause effects ranging from bile duct proliferation to distinct liver necrosis (Purchase, 1971). A survey of milk replacers used as animal feedstuffs found 46% of the samples were contaminated with aflatoxin $M_1$ (Mule et al., 1988).

A variety of physical and chemical techniques has been proposed to reduce the toxicity of aflatoxin-contaminated feed (Goldblatt and Dollear, 1979; Hagler et al., 1982; Yousef and Marth, 1986; Park et al., 1988; Jorgensen et al., 1990; Salmeron et al., 1990; Table 2. Residual concentrations of aflatoxin $M_1$ were detected on d 6, 10, and 12 in the milk of goats fed the control diet. There were no detectable levels of aflatoxin $M_1$ in the milk of goats fed diets containing hydrated sodium calcium aluminosilicate alone. The daily average concentration of aflatoxin $M_1$ in the milk of the aflatoxin-alone group ranged from .481 ppb on d 6 to .646 ppb on d 2. A statistically significant interaction resulting in the reduction of aflatoxin $M_1$ was observed for all time intervals when aflatoxin at 100 ppb was combined with 2% hydrated sodium calcium aluminosilicate in the diet of dairy goats. The daily mean aflatoxin $M_1$ residue ranged from a low of .093 ppb on d 2 to a high of .110 ppb on d 4 for the 2% hydrated sodium calcium aluminosilicate and aflatoxin combination. This combination resulted in daily mean aflatoxin $M_1$ residues in milk that were

Table 2. Effects of hydrated sodium calcium aluminosilicate (HSCAS) and aflatoxin (AF) on average daily concentrations of aflatoxin $M_1$ (ppb) in dairy goat milk

<table>
<thead>
<tr>
<th>Treatment</th>
<th>HSCAS, %</th>
<th>Period of analysis, d</th>
</tr>
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<tbody>
<tr>
<td>AF, ppb</td>
<td>2</td>
<td>4</td>
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<tr>
<td>0</td>
<td>0</td>
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<td>0</td>
<td>1</td>
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<tr>
<td>0</td>
<td>2</td>
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<tr>
<td>100</td>
<td>2</td>
<td>.094 ± .02*</td>
</tr>
<tr>
<td>100</td>
<td>1</td>
<td>.237 ± .13</td>
</tr>
<tr>
<td>100</td>
<td>0</td>
<td>649 ± 36</td>
</tr>
</tbody>
</table>

*aData reflect residue of naturally occurring aflatoxin in the diet. Data represent mean ± SE and n = 3.

bnd = not detected (detection limit .5 ppt).

*Means with asterisks are different ($P < .05$) from aflatoxin alone treated diet.
AFLATOXIN M₁ RESIDUE IN DAIRY GOAT MILK

Figure 2. Overall mean concentration Exp. 2 of aflatoxin M₁ in dairy goat milk during a 12-d exposure period to aflatoxin (AF) or AF + dietary hydrated sodium calcium aluminosilicate (HSCA). The aflatoxin M₁ residue in the control represents the naturally occurring level that was found in the feed. No aflatoxin M₁ was detected for hydrated sodium calcium aluminosilicate [1 and 2%] alone. Bars with different letters [a, b, c, d] are different (P < .05).

Samarajeewa et al., 1990; Schell et al., 1993). Modifications of mycotoxin contamination in feeds include: heat treatment (roasting and autoclaving), chemical inactivation (acid, alkali, aldehydes, oxidizing agents, and ammonia gases), solvent extraction (ethanol, acetone, isopropanol, or a mixture of solvents), and biological inactivation (yeast, molds, and certain bacteria). Other than ammoniation of contaminated cottonseed and peanuts, these methods have not been used on a commercial scale because of high costs, the need for special facilities, loss of important nutrients, and the questionable safety of chemical degradation products of aflatoxin. Economic and logistic considerations sometime restrict the use of conventional techniques to detoxify aflatoxin. The use of high-affinity adsorbents such as hydrated sodium calcium aluminosilicate in the diet of animals to eliminate or significantly diminish the toxicity and residual hazards of mycotoxins represents a very encouraging approach to the problem. Scientific data on the ability of hydrated sodium calcium aluminosilicate to decrease bioavailability of toxic agents such as the aflatoxin are of great importance.

In the present study, the potential of hydrated sodium calcium aluminosilicate to reduce aflatoxin M₁ in dairy goat’s milk was investigated in two experiments. Based on the data from the present study, it seems that addition of hydrated sodium calcium aluminosilicate, even at 4% of the diet, may not affect the nutritional quality of goat’s milk. In addition, 4% hydrated sodium calcium aluminosilicate in the diet did not significantly affect feed intake during this study. The combination of 4% hydrated sodium calcium aluminosilicate with aflatoxin at 200 ppb in the diet of dairy goats resulted in an overall reduction of 86.9% of aflatoxin M₁ residue, which was very close to the 81.3% observed with 2% hydrated sodium calcium aluminosilicate in combination with 100 ppb of aflatoxin in Exp. 2. Adding 1% hydrated sodium calcium aluminosilicate to the diet containing 100 ppb of aflatoxin resulted in a 51.9% reduction of aflatoxin M₁. Results from a previous study on dairy cows indicated that diets containing aflatoxin at 200 and 100 ppb had milk aflatoxin M₁ concentrations reduced by 27 and 42% with dietary concentrations of .5 and 1.0% hydrated sodium calcium aluminosilicate, respectively (Harvey et al., 1991b).

Presently, hydrated sodium calcium aluminosilicate is thought to absorb aflatoxin selectively during the digestive process, which renders much of the aflatoxin unavailable for absorption from the gastrointestinal tract (Kubena et al., 1990). Data of the present study indicate that the interaction of hydrated sodium calcium aluminosilicate and aflatoxin provides a potential protective mechanism against aflatoxin exposure. This is based on the reduced levels of aflatoxin M₁ excretion when aflatoxin is administered in combination with hydrated sodium calcium aluminosilicate. Results of this study provide further support for in vivo data in chickens (Phillips et al., 1988; Kubena et al., 1990), swine (Harvey et al., 1989), weanling pigs (Schell et al., 1993), turkeys (Kubena et al., 1991), lambs (Harvey et al., 1991a), and dairy cattle (Harvey et al., 1991b), suggesting that the bioavailability of aflatoxin is reduced by hydrated sodium calcium aluminosilicate (Harvey et al., 1991a). The reduced levels of aflatoxin M₁ excreted may be due to a strong bond formed between hydrated sodium calcium aluminosilicate and aflatoxin (Phillips et al., 1988) and, thus, reduced bioavailability of aflatoxin in the presence of hydrated sodium calcium aluminosilicate (Kubena et al., 1990).

In the current study, concentrations of aflatoxin M₁ in milk that exceeded the limit of .5 ppb permitted by the Food and Drug Administration for fluid milk were observed in goats of the aflatoxin-alone diets on the majority of days of Exp. 2; whereas in Exp. 1, aflatoxin M₁ concentrations were on the average threefold higher than the permitted level for milk. The addition of 1, 2, or 4% hydrated sodium calcium aluminosilicate reduced the aflatoxin M₁ residues in both Exp. 1 and 2 below the .5-ppb allowable level.

One area of concern is the ability of hydrated sodium calcium aluminosilicate to bind essential nutrients in the gastrointestinal tract (Phillips et al., 1990). It has been suggested that dietary hydrated sodium calcium aluminosilicate does not impair utilization of phosphorus in chickens (Chung and Baker, 1990). In another study (Chung et al., 1990) it was...
reported that hydrated sodium calcium aluminosilicate at 0.5 and 1.0% did not impair manganese, vitamin A, or riboflavin utilization. However, zinc utilization was reported to be reduced as a result of hydrated sodium calcium aluminosilicate ingestion at higher levels. Future experimentation needs to be carried out to provide answers or suggestions addressing such concerns.

Implications

Results from the present study have provided critically needed information regarding the ability of hydrated sodium calcium aluminosilicate to prevent or reduce the level of aflatoxin M1 residues in milk. Because milk is ultimately consumed by humans and animals the reduction of aflatoxin contamination in the milk could have an important impact on human and animal health.

Literature Cited


