ABSTRACT

STROUD, JILLIAN SUMMER. The effect of feed additives on aflatoxin in milk of dairy cows fed aflatoxin-contaminated diets. (Under the direction of Lon Whitlow and Brinton Hopkins.)

Sixty lactating Holstein cows were used in a replicated block experiment to determine the efficacy of eight feed additives to reduce the transfer of aflatoxin (**AF**) from feed to milk. Six cows were allocated to each treatment group and 12 to a control group. All cows were fed the same aflatoxin-contaminated total mixed ration (**TMR**) with either no additive (control) or one of eight additives at 0.5% of the TMR dry matter (**DM**). Milk samples were collected twice daily to evaluate changes in milk AF concentration, milk AF secretion (milk AF concentration × milk yield); and AF transfer from feed to milk (AF secretion as a percentage of AF intake). All changes were expressed as percentages and calculated relative to the control group which defined zero change. Four of the eight additives resulted in significant reductions ($P < 0.05$) ranging from 34.98 to 40.39% for milk AF concentration, 36.36 to 52.28% for milk AF secretion, and 34.45 to 48.44% for AF transfer. Dry matter intake (**DMI**) was significantly reduced ($P < 0.001$) by the consumption of AF, while milk production was not affected during the same time period. Neither DMI nor milk production were affected by the addition of treatment products to the diet when compared to control ($P > 0.05$).

THE EFFECT OF FEED ADDITIVES ON AFLATOXIN IN MILK OF DAIRY COWS FED AFLATOXIN-CONTAMINATED DIETS

by

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ABBREVIATIONS

AF	aflatoxin
AFB$_1$	aflatoxin B$_1$
AFM$_1$	aflatoxin M$_1$
AC	activated carbon
DMI	dry matter intake
EC	European Community
FDA	Food and Drug Administration
HSCAS	hydrated sodium calcium aluminosilicate
TMR	total mixed ration

LITERATURE REVIEW

Introduction

Aflatoxin (**AF**) is primarily produced by the fungi *Aspergillus flavus* and *Aspergillus parasiticus*. The major forms of aflatoxin responsible for contamination of feedstuffs include the forms B_1, B_2, G_1, and G_2. AFB_1 has been labeled as one of the most potent naturally occurring carcinogens (Varga and Téren, 1999). Aflatoxin can cause liver damage, reduce milk yield, feed intake, and overall performance. The biological effects of aflatoxin also include immunosuppression, mutagenicity, and teratogenicity (Betina, 1989). Contamination with aflatoxin can occur in common feedstuffs such as corn, cottonseed, and peanuts. This contamination can occur at all stages including field growth, storage, and feeding if conditions are suitable for growth of the fungus.

Due to the high intake of cows’ milk by humans, especially infants and children, many studies have been conducted measuring the carry-over, or transfer, of aflatoxin in the feed to the milk. Aflatoxin levels in both the feed and milk of dairy cows are regulated by the Food and Drug Administration (**FDA**). A maximum of 20 ppb of aflatoxin is permissible in feedstuffs consumed by lactating dairy cows, and no more than 0.5 ppb is tolerated in the milk for human consumption. Aflatoxin carryover to animal products also poses an economic liability and, thereby, drives the demand for a method to prevent aflatoxin contamination of feedstuffs and/or animal food products such as milk, eggs, and meat. One such method of prevention is the addition of adsorbents to dairy rations. By definition an adsorbent is a substance capable of attaching other substances to its surface without any chemical action. Adsorbent additives should bind the aflatoxin in the gastrointestinal tract and reduce absorption of aflatoxin by the animal.

This experiment was designed to use lactating Holstein cows, fed aflatoxin-contaminated diets with the addition of adsorbent additives to measure the reduction in aflatoxin concentration, secretion, and percent transfer into milk.

This literature review is divided into the following sections:

1. Aflatoxin Metabolites
2. Adsorbent Binding
    a. Phyllosilicates
        i. Bentonite
    b. Activated Carbon
    c. Other Potential Adsorbents
3. Carryover of aflatoxin from feed to milk

AFLATOXIN METABOLITES

Once ingested, aflatoxin can be converted to other toxic metabolites that are then secreted into animal products and tissues. The principal metabolite of the highly carcinogenic AFB_1 is what was first termed the “milk toxin” by de Iongh et al. (1964) and is now referred to as AFM_1. AFM_2 and AFM_4 have also been identified but less research has been conducted on these forms to date. Conversion of AFB_1 to AFM_1 involves a hydroxylation reaction at the 9a position of the AFB_1 heterocyclic molecule (Figure 1.). Although AFM_1 remains carcinogenic, it is less biologically active than AFB_1 (Purchase et al., 1967; Pong, 1971; Sinnhuber, 1974). In a study with rats fed a dietary level of 50 ppb, AFM_1 caused a 33% incidence of liver tumors, as compared to a 95% incidence for AFB_1 fed the same level (Hsieh et al., 1984).

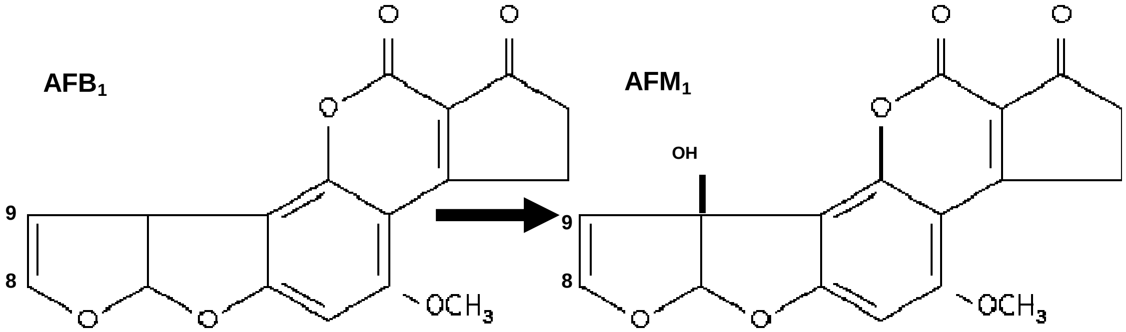

Figure 1. Conversion of AFB_1 to AFM_1 via hydroxylation.

ADSORBENT BINDING

Many mycotoxin binding agents have been studied and have resulted in varying success depending on the type(s) of mycotoxin present. These agents are added to the feed to reduce the absorption of mycotoxins by the gastrointestinal tract, thereby reducing accumulation of these toxins into animal tissues. As a feed additive, an adsorbent should possess certain qualities to be successful in reducing toxicity of the mycotoxin. Fundamentally, an adsorbent should bind the toxin in the gastrointestinal tract thereby reducing absorption by the animal and protect it against toxic effects. Understanding how the adsorbent is reducing toxicity is also important, because some binders, such as glucan polymers, may be more effective in binding aflatoxin than other mycotoxins. However, the mechanism of different binders with different mycotoxins has yet to be fully determined. It is also important for a producer to recognize which species and ages of animals may be more tolerant of mycotoxins and direct contaminated feeds to those animals or increase dilution of contaminated feedstuffs. The FDA does not allow the dilution of aflatoxin contaminated ingredients.

Mycotoxin contamination of feedstuffs is often unpreventable, and yet it may not be economically feasible for the farmer to discard all contaminated feed. This situation has led to the influx of research being done on the efficacy of different sequestering agents. Several potential binding agents are available including: clays, carbons, and glucan polymers. Studies testing the adsorbent capacity of these, as well as inorganic polymers, have shown there is also potential to bind aflatoxin as well as other mycotoxins (e.g. zearalenone, deoxynivalenol, T-2 toxin, ochratoxin A, etc. (Shreeve et al., 1978)).

As adsorbent agents, clays have somewhat of a complex nomenclature. Among the types or categories of clays are aluminas, silicates, phyllosilicates, zeolites, and bentonites. The differences between these clays may be chemical modification, processing, and the location of the clay within the mine from which it is taken. These differences can affect its chemical composition and secondary physical attributes (Diaz at al., 2002). In general, clay minerals are primarily layered silicates. Zeolites are tetrahedrons composed of SiO_4 and AlO_4 as the two foundational compounds with the metal atom at the center of the molecule. Zeolites act as ion exchange resins, so they are considered suitable for distinction of molecules by size, shape, and charge. Phyllosilicates are composed of layers of aluminum and silicon in a 1:1 or a 2:1 arrangement. These layers of silicates are comprised of chains of layers of divalent or trivalent cations held in *octahedral* coordination with oxygens or hydroxyls, or silicas that are *tetrahedrally* coordinated with oxygens and hydroxyls (Phillips, 1991).

There are three types of binding sites related to clays: 1) those located within interlayer channels, 2) located on the surface, and 3) uncoordinated metal ions located at edges of particles. Hydrated sodium calcium aluminosilicate (**HSCAS**) is a smectite classified as montmorillonite, a type of phyllosilicate, that contains calcium and protons which are exchanged against sodium ions. The only mechanism that has been published for specific chemisorption properties is that of HSCAS. Phillips et al. (1988) interpreted the significance of the beta-carbonyl system of AFB and AFG molecules for the formation of a complex with the ‘uncoordinated edge site’ of aluminum ions of HSCAS as reason for its more intense chemisorption quality.

Phyllosilicates

NovaSil is a smectite, one of the phyllosilicates, and has been shown to be effective in reducing AF absorption in chickens, turkey poults, lambs, goats, pigs, mink, and cattle; as well as alleviating some or all of the toxic effects on animal performance (Bonna et al., 1991, Davidson et al., 1987; Harvey et al., 1991; Kubena et al., 1991; Phillips et al., 1988). The amount of research performed on NovaSil, when compared to other products, reflects the great deal of interest and potential of this product. Phillips et al. (1988) reported on the *in vitro* binding properties of 38 different clay compounds, including aluminas, zeolites, silicas, phyllosilicates, and chemically modified phyllosilicates. The complex formed between NovaSil and AF in water was stable in pH 2, 7, and 10, and less than 10% was extracted, thus suggesting the formation of strong bonds between molecules. NovaSil was chosen to test *in vivo* in poultry based on its seemingly tight chemisorption with AF *in vitro*. NovaSil at 0.5% inclusion significantly reduced the growth inhibitory effect of feeding 7.5 mg AFB_1/kg feed in chickens.

Harvey et al. (1991) conducted a trial with lactating dairy cattle consuming 200 and 100 ppb AF with or without the addition of 0.5 or 1.0% phyllosilicate (a HSCAS), respectively. The 0.5% HSCAS added to the 200 ppb AF diet resulted in a 24%

reduction in secretion of AFM_1, while the 1.0% addition of HSCAS to the 100 ppb AF diet reduced AFM_1 secretion by 44%. These results suggest a possible dose response of the addition of adsorbent in relation to AF content of the feed, but this is confounded due to differences in AF concentrations of experimental diets.

Many studies with pigs have looked at performance parameters to determine the efficacy of phyllosilicates. In a study with growing pigs, HSCAS at 0.5% inclusion was added to an aflatoxin-contaminated diet containing 500-600 ng/g AFB_1 + AFB_2. Residues of AFM_1 were significantly reduced in liver, kidney, and muscle tissue (Beaver et al., 1990). Adding HSCAS in the typical range of 0.5% to 2.0% to aflatoxin-contaminated diets ranging up to 500+ ppb total AF, has shown production parameters, such as average daily gain or feed:gain ratio, to be comparable to those values seen with an aflatoxin free diet (Colvin et al., 1989; Harvey et al., 1989).

Bonna et al. (1991) conducted a study with mink consuming 34 or 102 ppb aflatoxin for 77 days with the addition of 0.5% HSCAS or 1.0% activated charcoal (**AC**). It was observed that the HSCAS prevented mortality, while AC reduced the incidence of mortality. The added adsorbents also reduced or eliminated the incidence of histopathologic liver lesions.

The majority of research regarding the addition of HSCAS to aflatoxin-contaminated diets has been done with chickens. HSCAS has been proven effective in reducing aflatoxicosis, maintaining body weight gains, feed:gain ratios (Huff et al., 1992; Kubena et al., 1993; and Ledoux et al., 1999) and decreasing the bioavailability of aflatoxin to the animal (Davidson et al., 1987).

The potential of phyllosilicates as adsorbent additives to aflatoxin-contaminated diets is promising, although results can vary depending on the total amount of mycotoxins present in the feed, and other mycotoxins may be in the feed which may not be adsorbed by the additives. Binding capacity is associated with amount of additive fed and may be affected by the composition of the feed. This may promote binding of dietary constituents, such as fiber and minerals to the clay instead of the (Schell et al., 1993).

Bentonite

Bentonites are also phyllosilicates characterized as having a layered crystalline microstructure which allows for the adsorption of other molecules. Bentonite consists mostly of montmorillonite, with the specific composition varying from one deposit to another, primarily due to the interchangeable ions, Na^+, K^+, Ca^{++}, and Mg^{++}. The ion that makes up the largest portion of the bentonite gives it its classification, i.e. sodium bentonite, potassium bentonite, etc. (Ramos et al., 1996).

The use of bentonites to adsorb aflatoxin was first examined by Masimango et al. (1978), who found that a 2% inclusion of several bentonites in a buffer solution resulted in adsorption of 400 µg AFB_1 ranging from 94 to 100%. The recovery of AFB_1 using a chloroform extraction procedure ranged from 5 to 23.3%, demonstrating there is variation in the adsorptive capacity of aflatoxin among bentonites.

Various adsorption tests have been conducted with bentonites in different types of solutions including milk, water, saline, blood serum and stomach fluid of pigs, and rumen fluid of cows. Dvorak (1989) found that 2.0% bentonite adsorbed 89% of AFM_1 in naturally contaminated whole milk which contained an initial AFM_1 content of 3 to 6 ppb.

Based on these *in vitro* studies, Lindemann et al. (1993) showed that the addition of sodium bentonite to growing swine diets containing 800 ppb AFB_1 resulted in improved average daily gains and feed intakes. The authors saw no further benefit of including sodium bentonite to the diet at a level exceeding 0.5%. Sodium bentonites have also been proven effective in reducing aflatoxicosis in broiler chickens. Araba and Wyatt (1991) demonstrated that sodium bentonite added to the diet at 1.0 and 0.5% effectively reduced the toxic effects of 5 ppm aflatoxin. The effects of aflatoxin on feed intake, body weight gains, liver weight and liver lipids were reduced by 52, 84, 74, and 90%, respectively with 1.0% sodium bentonite. At 0.5% bentonite, the same parameters were reduced by 68, 64, 74, and 17% respectively. These data show that 0.5% inclusion of sodium bentonite in the diet of broiler chickens may be more effective than 1.0%.

Diaz et al. (2004) conducted two experiments involving the efficacy of bentonites to reduce the transmission of aflatoxin in the feed to the milk of dairy cows. In experiment one, three sodium bentonites (Astra Ben20; Flow Guard, and Mycrosorb) were included in the aflatoxin-contaminated diet (containing 100 ppb AF) at 1.2% of the diet dry matter or 227 g/cow daily. Aflatoxin transmission from feed to milk was significantly reduced ($P < 0.001$) by 61, 65, and 50% for Astra Ben20, Flow Guard, and Mycrosorb, respectively.

Experiment two included the addition of four different adsorbent compounds, including Astra Ben20 (sodium bentonite), Red Crown bentonite (calcium bentonite), MTB-100 (glucomannan product) and AC-A (activated carbon). Both bentonite products were added to the diet at 1.2%, MTB-100 was added at 0.05%, and AC-A at 0.25%. The reductions in milk aflatoxin residues from the addition of the four adsorbents were 64.6, 31.4, 58.5, and 5.4%, respectively. All products except the activated carbon significantly reduced ($P < 0.01$) AFM_1 contamination in the milk.

Activated Carbon

Activated carbon, or charcoal, is a very porous, non-soluble powder formed by the pyrolysis of organic materials. It is a non-specific adsorbent and, therefore, will bind based on relative concentration of nutrients or compounds in the feed and not mycotoxins specifically. Carbon source, preparation methods, and physiochemical parameters, such as pore size distribution and surface area, can also determine the degree to which an activated carbon will bind the mycotoxin.

Several studies have reported variable results in the ability of AC to bind aflatoxin *in vitro* and *in vivo*. These studies have been done predominantly with poultry (Kubena, 1990; Jindal, 1994; Ademoyero & Dalvi, 1983; Edrington, 1996), but there has also been work done with mink (Bonna, 1991) and cattle (Galvano, 1996; Diaz, 2004). Edrington et al. (1996) found that AC was effective in reducing urinary excretion of AFM_1 in turkey poults when dosed concomitantly with AFB_1. The sorbent was added to the AF-contaminated diet (containing 0.75 mg/kg BW AFB_1) at 0.5%, the reduction in weight gain was no different for the poults that received aflatoxin only or those receiving either of two ACs. Two sources of AC were used in this experiment to see if different carbon sources would have different binding abilities.

Twelve late lactation Fresian cows consumed 11.28 µg/kg feed AFB_1 in naturally contaminated corn meal for one week (Edrington, 1996). The following week, the cows received the same diet plus the addition of 2.0% AC (CAC_1, CAC_2) or HSCAS. The three adsorbents resulted in the carryover of AFB_1 to AFM_1 in the milk of 0.34, 0.27, and 0.41 % (HSCAS, CAC_1, CAC_2, respectively). The lesser effectiveness of the CAC_2 may have been due to the anti-caking property of this AC, resulting in unsuccessful pelleting of the feed. With the intake of AFB_1 approximately 55 µg/d, the AFM_1 content of the milk, with or without the addition of adsorbents, resulted in levels that remained below the European countries (**EC**) tolerance level of 0.05 ppb. This is important to note due to the small amount of aflatoxin present and the inclusion of adsorbents at 2.0%, which is higher than used in many studies.

Other Potential Adsorbents

Organic polymers, such as cellulose, glucomannans and peptidoglycans derived from yeast cell walls, have potential as aflatoxin adsorbents. Esterified glucan polymer, extracted from yeast cell wall, has been effective in reducing the deleterious effects caused by aflatoxin in broiler chicks (Basmacioglu et al., 2005), and has also reduced milk aflatoxin residues in dairy cows (Diaz et al., 2004). Polyvinylpyrrolidone (**PVP**) is a synthetic water soluble polymer that is reported to bind aflatoxin *in vitro* (Alegakis et al., 1999). Celik et al. (2000) showed PVP reduced the affects of aflatoxin in chicks.

CARRYOVER OF AFLATOXIN FROM FEED TO MILK

The concern for contamination of milk by AFM_1 prompted several studies with the objective to establish a percentage transfer of aflatoxin from feed to milk of dairy cows. A range of 0 to 4% reported by Sieber and Blanc (1978) was described in a review by van Egmond (1989) showing the relationship between AFB_1 intake and AFM_1

secretion in the milk. The data of eight different studies was used to create the regression equation $y = -2.55 + 0.84x$ ($r^2 = 0.73$; $n = 43$) presented in the review.

Patterson et al. (1980) found an average of approximately 2.2% of the ingested AFB_1 was converted to and secreted as AFM_1 in the milk in cows receiving 10 ppb AFB_1 in the diet daily. Lafont et al. (1980) conducted an experiment with early and late lactation cows, receiving 0.09, 0.18, 0.86, or 2.58 mg AFB_1 daily. It was concluded in this study that the percent AFB_1 secreted as AFM_1 in the milk ranged from 0.14 to 0.34%, with an average of 0.22% in the late lactation cows, and a range of 0.66 to 0.95%, with an average of 0.78% was seen in cows producing at least 20 L of milk per day. Although milk yield for cows in the study conducted by Patterson et al. (1980) did not differ from those in the early lactation cows in the Lafont et al. (1980) study, the experimental transfer percent differed by 0.56%. A definite transfer percent from these two studies cannot be determined due to the variation observed.

Later work conducted by Frobish et al. (1986) determined a mean percent of daily AFB_1 intake that was secreted as AFM_1 in milk of 1.74. The percent transfer for individual cows ranged from 0.4 to 3.68, with the 95% confidence interval associated with the mean being 1.47 to 2.02%. Based on this study, the mean percent transfer was unaffected by concentration of aflatoxin in the feed, but a significant positive effect ($P < 0.01$) was seen with increased milk production. Higher producing cows resulted in a 2.14% transfer compared to a 1.35% transfer observed in the lower producing cows. However, the concentration of AFM_1 in the milk was not affected by milk production. Frobish et al. (1986) also looked at differences in source of aflatoxin contamination in a second trial using similar amounts of aflatoxin in naturally contaminated cottonseed meal and corn (44 and 49 µg/kg, respectively). The percent of AFM_1 secreted into the milk was significantly affected (*P* < 0.02) by source of contamination with 1.73% transfer for the cottonseed meal and 1.32% transfer for the corn. Again, milk aflatoxin concentration was not different for either contaminant source.

In a further attempt to help narrow the range of percentage transfer of aflatoxin from feed to milk, the data from 14 studies were compiled and analyzed. All values reported were converted to a concentration (ppb) for both aflatoxin intake and aflatoxin secretion. To calculate this conversion, an assumed efficiency ratio of 0.59 kg milk/kg DMI was used due to the lack of intake data reported in each study (Britt et al., 2003).

Analysis of the data was done using PROC REG (SAS®, 2001) with intercept set to zero. The data resulted in the equation AFM_1 ppb = 0.0092 AFB_1 ppb; $R^2 = 0.596$, n = 83) (Figure 2.). The data were also divided based on aflatoxin intake of either less than or greater than 150 ppb. It can be observed that at higher concentrations of aflatoxin intake, milk aflatoxin secretion is more variable even though the R^2 value is similar and the equation is the same to that of the total data set ($R^2 = 0.5974$; AFM_1 ppb = 0.0092 AFB_1 ppb) (Figure 3.). The number of data points differ for the data set with concentrations > 150 ppb compared to the total data set (n = 20 vs. n = 83, respectively). At lower aflatoxin intakes, which are concentrations more commonly observed in the field, a more linear response is seen. The lower concentration data set resulted in the equation AFM_1 ppb = 0.0104 AFB_1 ppb; $R^2 = 0.656$, n = 65 (Figure 4.). Although these values fall within the range reported by Sieber and Blanc (1978), they are on lower end of the range. Approximately a 1.0% transfer of AFB_1 to AFM_1 may be more representative of the data reported in published trials based on the results of this analysis.

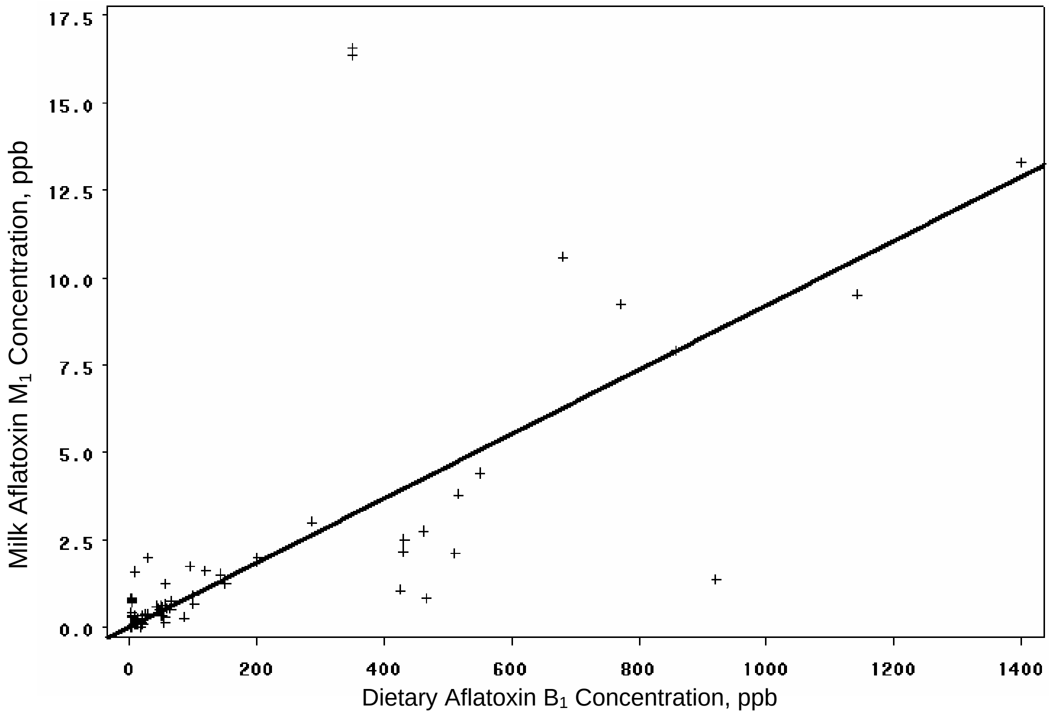

Figure 2. Relationship between dietary aflatoxin concentration (AFB_1) and aflatoxin concentrations (AFM_1) in milk.

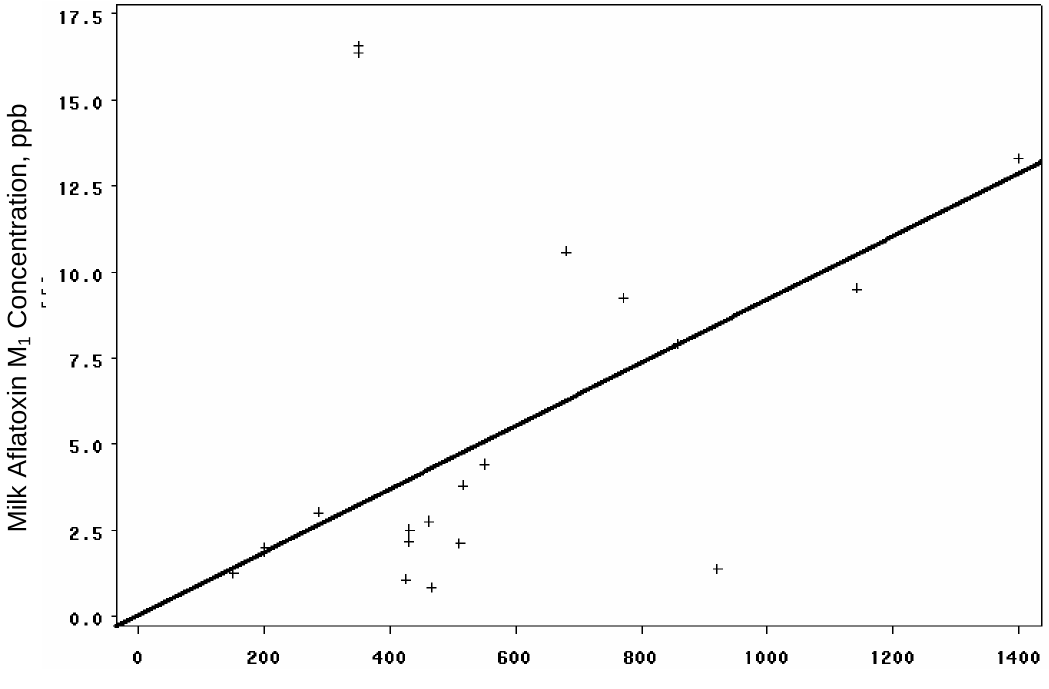

Figure 3. Relationship between high dietary aflatoxin concentrations (>150ppb AFB_1) and aflatoxin concentrations (AFM_1) in milk.

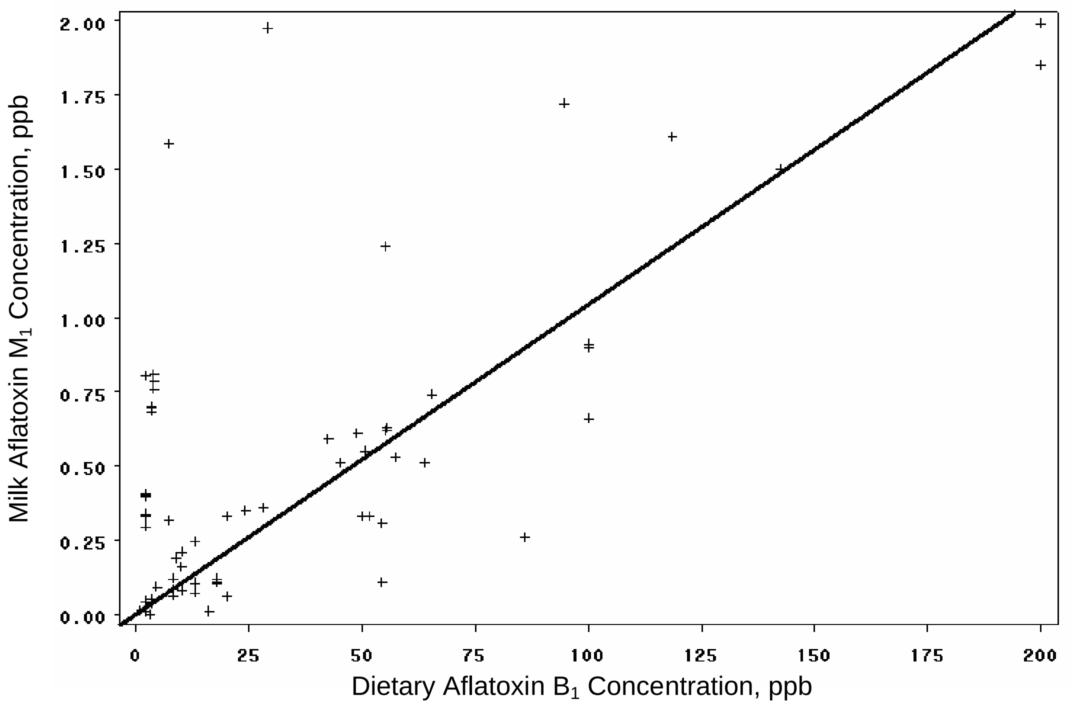

Figure 4. Relationship between low dietary aflatoxin concentrations (<150ppb AFB_1) and aflatoxin concentrations (AFM_1) in milk.

Determining an average percent transfer of aflatoxin is a multi-faceted evaluation which makes it difficult to compare and group studies. Several aspects including the variation in reported aflatoxin intake, dosed aflatoxin vs. naturally contaminated feed, source of contaminated feed, cow variation, and difficultly in measuring aflatoxin in the feed all contribute to the overall complexity of the carryover equation.

The differences in reported aflatoxin intakes make it difficult to form paralleled comparisons between two or more experiments. Feed and milk aflatoxin is often reported as amounts per day, total amounts, and/or concentrations. To best analyze the data, all units should be on the same basis. This becomes difficult because many papers do not report dry matter intake (**DMI**) or milk production for cows on trial; therefore, if converting amounts to concentrations, an assumed DMI and milk yield must be used in the calculation thus reducing accuracy of reported values.

The current review found that of the 14 studies, three dosed cows with pure AFB_1. Each of these three studies contributed to the overall outliers observed (Applebaum et al., 1982, Stubblefield et al., 1983, and Polan et al., 1974). This is evidence that there are possible interactions among toxic metabolites and the effects of impure vs. pure aflatoxin may differ. Using pure AFB1 is not indicative of actual aflatoxin intakes by animals nor does it represent the growth of aflatoxin in the feed. Source of contamination, such as cottonseed meal vs. corn, resulted in significant differences in estimate of carryover when conducted in the same study (Frobish et al., 1986). The sources of aflatoxin, contamination cited from published work used for this review, varied considerably as illustrated in Table 1.

Table 1. Percent reductions in milk aflatoxin concentration, milk aflatoxin secretion, and milk aflatoxin transfer for each treatment.

Study Identification	Aflatoxin Source	*n* =
Munksgaard et al., 1987	Naturally contaminated cottonseed cakes	12
Harvey et al., 1991	Fermented rice	3
Diaz et al., 2004	Naturally contaminated corn grain	16
Galvano et al., 1996	Naturally contaminated corn meal	12
Veldman, 1992	Naturally contaminated groundnut meal	9
Stubblefield et al., 1983	Dosed AFB_1	2
Applebaum et al., 1982	Dosed pure and impure AFB_1	10
Fremy and Quillardet, 1985	Naturally contaminated groundnut meal	8
Sassahara et al., 2005	NA*	NA*
Shreeve et al., 1979	Naturally contaminated wheat and barley	2
Polan et al., 1974	Dosed AFB_1	4
Patterson et al., 1980	Protein balancer, barley-mix, and dairy nuts	6
Allcroft and Roberts, 1968	Naturally contaminated groundnut meal	NA*
Frobish et al., 1986	Naturally contaminated cottonseed meal	32

** NA = Not available*

Cow to cow variation affects the reported means of aflatoxin transfer because of the broad range that is seen from cow to cow, day to day, and from one milking to the next. Animal experiments are expensive and time consuming, and therefore observations are of few animals over a short period. Many experiments may have only used two cows to evaluate aflatoxin carryover, which greatly reduces the power of the results. It has been hypothesized that some of the cow variation can stem from the condition of the udder, under the assumption that AFM_1 diffuses from blood into the milk. Poor udder health may affect the permeability of the alveolus cell membranes of the mammary gland, and therefore affect the amount of AMF_1 that is diffused across the membrane and into secreted milk (Veldman, 1992).

Yet another obstacle in evaluating aflatoxin transfer from feed to milk is the dilemma in precise sampling of contaminated feed for aflatoxin content. Variation in the test procedure is the sum of sampling, sample preparation, and analytical variances (Whitaker et al., 2005). Of the 14 studies evaluated, the years in which these experiments were conducted ranged from 1968 to 2005. Sampling techniques and accuracy in analytical procedures are sure to have been improved over this 36 year period.

SUMMARY

The regulatory limit of 0.5 ppb AFM_1 in milk will be exceeded if aflatoxin levels in the feed exceed 50 ppb assuming a transfer of 1.0%. The lower limit set by EC at 0.05 ppb AFM_1 will be exceeded when dietary concentrations of aflatoxins are above a level of 5 ppb aflatoxin. In areas where aflatoxin contamination naturally occurs and during seasons where weather is conducive to aflatoxin growth, this will become a hardship for producers in these geographical regions. Based on the review of data, however, it is impossible to set an exact percentage transfer of aflatoxin from feed to milk, and 1.0% may be too low for some cows. The degree of variability of aflatoxin transfer is extremely large. It will be important for the dairy industry to determine a way to quickly and accurately measure aflatoxin content of the feed, prevent aflatoxin contamination of the feed, and prevent or reduce aflatoxin transfer from feed to milk.

LITERATURE CITED

Ademoyero, A.A., and R.R. Dalvi. 1983. Efficacy of activated charcoal and other agents in the reduction of hepatotoxic effects of a single dose of aflatoxin B1 in chickens. Toxicol. Lett. 16(1-2):153-157.

Alegakis, A.K., A.M. Tsatsakis, M.I. Shtilman, D.L. Lysovenko, and I.G. Vlachonikolis. 1999. Deactivation of mycotoxins. I. An in vitro study of zearalenone adsorption on new polymeric adsorbents. J. Environ. Sci. Health B. 34(4):633-644.

Allcroft, R., and B.A. Roberts. 1968. Toxic groundnut meal: The relationship between aflatoxin AFB_1 intake by cows and excretion of aflatoxin AFM_1 in milk. Vet. Rec. 82:116-118.

Applebaum, R.S., R.E. Brackett, D.W. Wiseman, and E.H. Marth. 1982. Responses of dairy cows to dietary aflatoxin: feed intake and yield, toxin content, and quality of milk of cows treated with pure and impure aflatoxin. J. Dairy Sci. 65(8):1503-1508.

Araba, M., and R.D. Wyatt. 1991. Effects of sodium bentonite, hydrated sodium calcium alumino-silicate (NovaSil), and ethacal on aflatoxicosis in broiler chickens. Poult. Sci. 70:S6. (Abstr.)

Basmacioglu, H., H. Oguz, M. Ergul, R. Col, and Y.O. Birdane. 2005. Effect of dietary esterified glucomannan on performance, serum biochemistry and haematology in broilers exposed to aflatoxin. Czech J. Anim. Sci. 50:31-39.

Beaver, R.W., D.M. Wilson, M.A. James, K.D. Haydon, B.M. Colvin, L.T. Sangster, A.H. Pikul, and J.D. Groopman. 1990. Distribution of aflatoxins in tissues of growing pigs fed an aflatoxin-contaminated diet amended with a high affinity aluminosilicate. Vet. Hum. Toxicol. 32(1):16-18.

Betina, V. 1989. Structure-activity relationships among mycotoxins. Chem. Biol. Interact. 71(2-3):105-146.

Bonna, R.J., R.J. Aulerich, S.J. Bursian, R.H. Poppenga, W.E. Braselton, and G.L. Watson. 1991. Efficacy of hydrated sodium calcium aluminosilicate and activated charcoal in reducing the toxicity of dietary aflatoxin to mink. Arch. Environ. Contam. Toxicol. 20(3):441-447.

Britt, J.S., R.C. Thomas, N.C. Speer, and M.B. Hall. 2003. Efficiency of converting nutrient dry matter to milk in Holstein herds. J. Dairy Sci. 86:3796-3801.

Celik, I., H. Oguz, O. Demet, H.H. Donmez, M. Boydak, and E. Sur. 2000. Efficacy of polyvinylpolypyrrolidone in reducing the immunotoxicity of aflatoxin in growing broilers. Br. Poult. Sci. 41:430-439.

Colvin, B.M., L.T. Sangster, K.D. Haydon, R.W. Beaver, and D.M. Wilson. 1989. Effect of a high affinity aluminosilicate sorbent on prevention of aflatoxicosis in growing pigs. Vet. Hum. Toxicol. 31(1):46-48.

Davidson, J.N., J.G. Babish, K.A Delaney, D.R. Taylor, and T.D. Phillips. 1987. Hydrated sodium calcium aluminosilicate decreases the bioavailability of aflatoxin in the chicken. Poult. Sci. 66:89. (Abstr.)

De Iongh, H., R.O. Vles, van Peltj. 1964. Milk of Mammals fed an Aflatoxin-contaminated diet. Nature. 202:466-467.

Diaz, D.E., W.M. Hagler Jr., B.A. Hopkins, and L.W. Whitlow. 2002. Aflatoxin binders I: in vitro binding assay for aflatoxin B1 by several potential sequestering agents. Mycopathologia. 156(3):223-226.

Diaz, D.E., W.M. Hagler Jr., J.T. Blackwelder, J.A. Eve, B.A. Hopkins, K.L. Anderson, F.T. Jones, and L.W. Whitlow. 2004. Aflatoxin binders II: reduction of aflatoxin M1 in milk by sequestering agents of cows consuming aflatoxin in feed. Mycopathologia. 157(2):233-241.

Dvorak, M. 1989. Ability of bentonite and natural zeolite to adsorb aflatoxin from liquid media. Vet. Med. (Praha). 34(5):307-316.

Edrington, T.S., A. B. Sarr, L.F. Kubena, R.B. Harvey, and T.D. Phillips. 1996. Hydrated sodium calcium aluminosilicate (HSCAS), acidic HSCAS, and activated charcoal reduce urinary excretion of aflatoxin M1 in turkey poults. Lack of effect by activated charcoal on aflatoxicosis. Toxicol. Lett. 89:115-122.

Fremy, J.M., and P. Quillardet. 1985. The “carry over” of aflatoxin into milk of cows fed ammoniated rations: use of an HPLC method and a genotoxicity test for determining milk safety. Food Addit. Contam. 2:201-207.

Frobish, R. A., B.D Bradley, D.D. Wagner, P.E. Long-Bradley, and H. Hairston. 1986. Aflatoxin residues in milk of dairy cows after ingestion of naturally contaminated grain. J. Food Prot. 49: 781-785.

Galvano, F., A. Pietri, T. Bertuzzi, G. Fusconi, M. Galvano, A. Piva, and G. Piva. 1996a. Reduction of carryover of aflatoxin from cow feed to milk by addition of activated carbons. J. Food Prot. 59: 551-554.

Harvey, R.B., L.F. Kubena, T.D. Phillips, D.E. Corrier, and W.E. Huff. 1989a. Prevention of aflatoxicosis by addition of hydrated sodium calcium aluminosilicate to the diets of growing barrows. Am. J. Vet. Res. 50:416-420.

Harvey, R. B., T.D. Phillips, J.A. Ellis, L.F. Kubena, W.E. Huff, and H.D. Petersen. 1991. Effects on aflatoxin Ml residues in milk by addition of hydrated sodium calcium aluminosilicate to aflatoxin-contaminated diets of dairy cows. Am. J. Vet. Res. 52: 1556-1559.

Hsieh, D.P., J.M. Cullen, and B.H. Ruebner. 1984. Comparative hepatocarcinogenicity of aflatoxins B1 and M1 in the rat. Food Chem. Toxicol. 22(12):1027-1028.

Huff, W.E., L.F. Kubena, R.B. Harvey, and T.D. Phillips. 1992. Efficacy of hydrated sodium calcium aluminosilicate to reduce the individual and combined toxicity of aflatoxin and ochratoxin A. Poult. Sci. 71:64-69.

Jindal, N., S.K. Mahipal, and N.K. Mahajan. 1994. Toxicity of aflatoxin B1 in broiler chicks and its reduction by activated charcoal. Res. Vet. Sci. 56(1):37-40.

Kubena, L.F., R.B. Harvey, W.E. Huff, D.E. Corrier, T.D. Phillips, and G.E. Rottinghaus. 1990. Efficacy of a hydrated sodium calcium aluminosilicate to reduce the toxicity of aflatoxin and T-2 toxin. Poult. Sci. 69(7):1078-1086.

Kubena, L.F., W.E. Huff, R.B. Harvey, A.G. Yersin, M.H. Elissalde, D.A. Witzel, L.E. Giroir, T.D. Phillips, and H.D. Peterson. 1991. Effects of a hydrated sodium calcium aluminosilicate on growing turkey poults during aflatoxicosis. Poult. Sci. 71:1823-1830.

Kubena, L.F., R.B. Harvey, T.D. Phillips, and B.A. Clement. 1993. Effect of hydrated sodium calcium aluminosilicates on aflatoxicosis in broiler chicks. Poult. Sci. 72(4):651-657.

Ledoux, D.R., G.E. Rottinghaus, A.J. Bermudez, and M. Alonso-Debolt. 1999. Efficacy of a hydrated sodium calcium aluminosilicate to ameliorate the toxic effects of aflatoxin in broiler chicks. Poult. Sci. 78(2):204-210.

Lindemann, M.D., D.J. Blodgett, E.T. Kornegay, and G.G. Schurig. 1993. Potential ameliorators of aflatoxicosis in weanling/growing swine. J. Anim. Sci. 71:171-178.

Masimango, N., J.L. Ramaut, and J. Remacle. 1977. Production of aflatoxin B1 in vitro as a function of various culture conditions. Ann. Nutr. Aliment. 31(4-6):583-605.

Munksgaard, L., J. Larsen, and H. Werner. 1987. Carry-over of aflatoxin from cows feed to milk and milk products. Milchwissenschaft. 42:165-167.

Patterson, D.S., E.M. Glancy, and B.A. Roberts. 1980. The 'carry over' of aflatoxin M1 into the milk of cows fed rations containing a low concentration of aflatoxin B1. Food Cosmet. Toxicol. 18(1):35-37.

Phillips, T. D., L.F. Kubena, R.B. Harvey, D.R. Taylor, and N.D. Heidelbaugh. 1988. Hydrated sodium calcium aluminosilicate: A high affinity sorbent for aflatoxin. Poult. Sci. 67:243-247.

Phillips, T.D., B.A. Clement, and D.L. Park. 1991. Approaches to Reductions of Aflatoxins in Foods and Feeds.

Phillips, T.D., B.A. Sarr, B.A. Clement, L.F. Kubena, and R.B. Harvey. 1991. Prevention of aflatoxicosis in farm animals via selective chemisorption of aflatoxin. Pages 223-237 in Mycotoxins, Cancer and Health. Vol. 1. G.A. Bray and D.H. Ryan, ed. Lousisiana State University Press, Baton Rouge, LA.

Polan, C.E., J.R. Hayes, and T.C. Campbell. 1974. Consumption and fate of aflatoxin B1 by lactating cows. J. Agric. Food Chem. 22(4):635-638.

Pong, R.S., and G.N. Wogan. 1971. Toxicity and biochemical and fine structural effects of synthetic aflatoxins M 1 and B 1 in rat liver. J. Natl. Cancer Inst. 47(3):585-592.

Purchase, IF. 1967. Acute toxicity of aflatoxins M1 and M2 in one-day-old ducklings. Food Cosmet Toxicol. 5(3):339-342.

Ramos, A., J. Fink-Gremmels, and E. Hernandez. 1996. Prevention of toxic effects of mycotoxins by means of nonnutritive adsorbent compounds. J. Food Prot. 59:631-641.

SAS. 2001. SAS/STAT User's Guide: Statistics, Version 8.2, SAS Institute Inc., Cary, NC.

Sassahara, M., D. Pontes Netto, and E.K. Yanaka. 2005. Aflatoxin occurrence in foodstuff supplied to dairy cattle and aflatoxin M1 in raw milk in the North of Parana state. Food Chem. Toxicol. 43(6):981-984.

Schell, T.C., M.D. Lindemann, E.T. Kornegay, and D.J. Blodgett. 1993. Effects of feeding aflatoxin-contaminated diets with and without clay to weanling and growing pigs on performance, liver function, and mineral metabolism. J. Anim. Sci. 71(5):1209-1218.

Shreeve, B.J., D.S. Patterson, and B.A. Roberts. 1979. The 'carry-over' of aflatoxin, ochratoxin and zearalenone from naturally contaminated feed to tissues, urine and milk of dairy cows. Food Cosmet. Toxicol. 17(2):151-152.

Sieber, R. and B. Blanc. 1978. Zur Ausscheidung von Aflatoxin m1 in die milch und dessen vorkommen in milch und milchprodukten – eine Literaturübersicht. Mitt. Gebiete Lebensm. Hyg., 69:477-491.

Sinnhuber, R.O., D.J. Lee, J.H. Wales, M.K. Landers, and A.C. Keyl. 1974. Hepatic carcinogenesis of aflatoxin M1 in rainbow trout (Salmo gairdneri) and its enchancement by cyclopropene fatty acids. J. Natl. Cancer Inst. 53(5):1285-1288.

Stubblefield, R.D., A.C. Pier, J.L. Richard, and O.L. Shotwell. 1983. Fate of aflatoxins in tissues, fluids, and excrements from cows dosed orally with aflatoxin B1. Am. J. Vet. Res. 44(9):1750-1752.

Van egmond, H.P. 1989. Aflatoxin M1: occurrence, toxicity, regulation. Pages 11-55 in Mycotoxins in Dairy Products. Van Egmond, ed., Elsevier Sci. Pub. Co., Ltd., New York, NY.

Varga, J. and J. Teren. 1999. Recent progress in mycotoxin research. Acta. Microbiol. Immunol. Hung. 46:233-243.

Veldman, A. 1992. Effect of sorbentia on carry-over of aflatoxin from cow feed to milk. Milchwissenschaft. 47:777-780.

Whitaker, T.B., A.B. Slate, and A.S. Johansson. 2005. Sampling feeds for mycotoxin analysis. Pages 1-23 in The Mycotoxin Blue Book. D.E. Diaz, ed. Nottingham Publishing Press, Nottingham, UK.

Efficacy of feed additives to bind aflatoxin. *By Stroud et al.* Eight adsorbent products were evaluated to determine the percent reduction in milk aflatoxin concentration, secretion, and transfer that occurred when added at 0.5% of diet dry matter to an aflatoxin-contaminated diet consumed by Holstein cows. Four of the eight products resulted in significant positive values and were, therefore, deemed effective when compared to the control cows to which no adsorbent product was fed.

RUNNING HEAD: POTENTIAL ADSORBENTS OF AFLATOXIN

The Effect of Feed Additives on Aflatoxin in Milk of Dairy Cows Fed Aflatoxin-contaminated Diets.

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ABSTRACT

Sixty lactating Holstein cows were used in a replicated block experiment to determine the efficacy of eight feed additives to reduce the transfer of aflatoxin (**AF**) from feed to milk. In each replicate, six cows were allocated to each treatment group and 12 to a control group. All cows were fed the same aflatoxin-contaminated total mixed ration (**TMR**) for 11 d with either no additive (control) or one of eight additives included at 0.5% of the TMR dry matter (**DM**) during the last 6 d of the replicate. Milk samples were collected when additives were fed or not fed to evaluate changes in milk AF concentration, milk AF secretion (milk AF concentration × milk yield); and AF transfer from feed to milk (AF secretion as a percentage of AF intake). All changes were normalized to the control group and expressed such that a positive percentage indicated a reduction in AF values associated with feed additive inclusion. Four of the eight additives resulted in significant reductions ($P < 0.05$) ranging from 34.98 to 48.9% for milk AF concentration, 36.36 to 52.28% for milk AF secretion, and 34.45 to 48.44% for AF transfer. Dry matter intake (**DMI**) was significantly reduced ($P < 0.001$) by the consumption of AF, while milk production was not affected during the same time period. Neither DMI nor milk production were affected by the addition of treatment products to the diet when compared to control ($P > 0.05$).

(**Key words:** adsorption, aflatoxin, dairy)

INTRODUCTION

Feeding aflatoxin-contaminated diets to lactating animals results in secretion of aflatoxin M_1 (**AFM_1**) in the milk (van Egmond and Paulsch, 1986). AFM_1 is the metabolite formed via a hydroxylation reaction from the highly carcinogenic aflatoxin B_1

present in the feed (**AFB$_1$**) (Eaton et al., 1994). While the potency of AFM$_1$ is less than AFB$_1$, it remains a carcinogen. The carryover of aflatoxin from feed to milk is of great concern due to the large consumption of milk and milk products by humans, especially infants and children. The Food and Drug Administration (**FDA**) regulates the concentration of aflatoxin in both feed and milk of dairy cows. The concentration of aflatoxin in milk is not permissible above 0.5 ppb (Code of Federal Regulations Part 109 and 509).

Several *in vitro* studies have shown that adsorbent products including clays (typically hydrated sodium calcium aluminosilicates), activated carbons, and yeast products are effective in binding aflatoxin (Diaz et al., 2002; Maryamma et al., 1991; Phillips et al., 1988). However, the effectiveness of products *in vitro* may not predict effectiveness *in vivo* (Diaz et al., 2004; Lemke et al., 2001). The objective of this study was to evaluate the efficacy of different adsorbent products in reducing milk aflatoxin concentration, secretion, and aflatoxin transfer *in vivo* using a standardized procedure.

MATERIALS AND METHODS

Diet

Naturally contaminated corn was used as the aflatoxin source for the total mixed ration (**TMR**). Corn oil and molasses were added to the contaminated corn to eliminate the hazard of inhaling aflatoxin, and to contain the fines where much of the aflatoxin is concentrated in the feed. The contaminated corn was ground and mixed thoroughly to promote proper distribution of aflatoxin in the corn. The corn was then blended with the TMR in a mixer wagon. At each feeding, one of the eight different adsorbents was individually blended into the appropriate amount of TMR for each feeding group. Each treatment diet was blended using a DataRanger (American Calan Inc., Northwood, NH) mixer and fed to cows through Calan (American Calan Inc., Northwood, NH) feeding stations. After each treatment ration was fed, the mixer was flushed with 28 kg silage to avoid cross contamination of treatment products. Cows consumed 170 ppb total aflatoxin which was calculated based on the milk aflatoxin concentration of the control cows and assuming 1.7% transfer of aflatoxin from feed to milk (Frobish et al., 1986).

Experimental Design

Sixty lactating Holstein cows from the Piedmont Research Station in Salisbury, NC were randomly assigned to nine treatment groups. The treatment groups were 1) MTB-100 (Alltech, Inc., Nicholasville, KY); 2) UltraSorb (Micron Bio-systems, Inc., Buena Vista, VA); 3) Mexsil (Karluis Enterprises, Queretaro, Mexico); 4) NovaSilplus (Trouw Nutrition, Highland, IL); 5) Toxynil+ (INVE Technologies, Dendermonde, Belgium); 6) Condition Ade (Oil·Dri Corporation, Chicago, IL); 7) Astra Ben 20A (Prince Agri Products, Inc., Quincy, IL); 8) Milbond-TX (Milwhite, Inc., Brownsville, TX); and 9) Control (no additive). MTB-100, UltraSorb, and Toxynil+ were composed of yeast and silicates, while the remaining adsorbents were composed of silicates only. The adsorbents were added to the diet at 0.5% of the diet dry matter or approximately 100g/cow/day. The experiment was designed as a replicated block with six cows allocated to each treatment group and twelve cows in the control group, blocked by both milk production and parity (1or >1). In replicate two, cows were reallocated so that no cow remained on the same treatment and remained blocked by parity and milk production. The lower producers of the herd were used for the experiment, with an average milk production of 25.8 kg/day.

For each replicate, cows were fed an aflatoxin-contaminated TMR for five days with no additive in the diet. For the following six days, the aflatoxin-contaminated TMR was fed with the addition of the treatment additive. Evening (1400h) and the following morning (0200h) milk samples were taken at day one to establish a baseline concentration of aflatoxin in milk prior to feeding aflatoxin-contaminated diets. Only a trace amount (average = 0.007 ppb) was detected. Milk samples were taken at the end of the periods of aflatoxin consumption, days 5 and 15, and at the end of aflatoxin plus additive consumption, days 10,11, 20, and 21 (Figure 1.). Milk samples were frozen until analyses. Samples of composite milk from all experimental cows was collected and analyzed daily so that milk was discarded until AFM$_1$ was cleared from the milk. Feed samples were collected daily to determine diet dry matter. Diets were formulated to meet the nutrient requirements for the group average milk production (NRC, 2001). To ensure total intake of the additive, feed consumption was measured and then diets were allocated to minimize orts, resulting in a DMI of approximately 23 kg/cow daily.

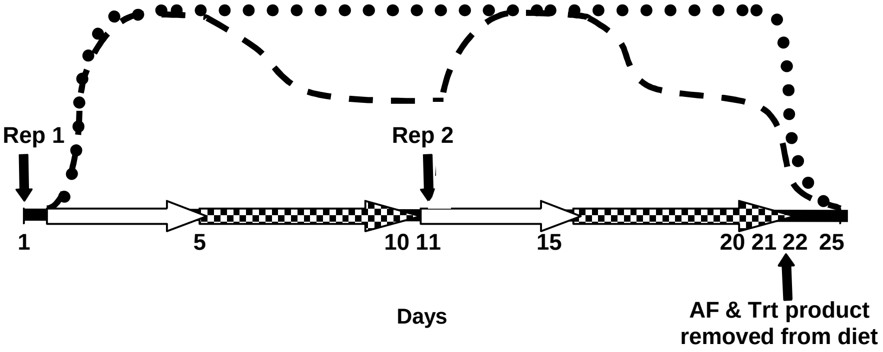

Figure 1. Timeline illustrating the two replicates of the experiment, periods of AF intake ▭, AF plus treatment product ▩, milk sampling days, the theoretical AFM$_1$ concentration in response to feeding AF …, and the theoretical concentration following the addition of an adsorbent product to an AF-contaminated diet ---.

AFM$_1$ Analysis

Milk samples were analyzed for AFM1 using HPLC and VICAM affinity columns as described by Viacom L.P. Instruction Manual # 6M-MC 9512-3 (Viacom L.P., Watertown, MA). Milk samples were composited by day then defatted prior to analysis. A 35 ml sample of defatted milk was then passed through the AFM$_1$ affinity column and filtered. Water was passed through the column and AFM$_1$ eluted and collected in a scintillation vial. The eluate was analyzed by HPLC. Concentrations of AFM$_1$ were determined relative to a quantitative AFM$_1$ standard.

Statistical Analysis

The efficacy of each treatment additive was evaluated independently based on three measures of effectiveness. These measures include the reduction in milk aflatoxin concentration, the reduction in milk aflatoxin secretion (calculated as milk aflatoxin concentration × milk yield), and reduction in aflatoxin transfer (calculated as aflatoxin secretion as a percentage of aflatoxin intake). All treatment data were normalized to control data, and then evaluated independently for differences from zero. Significant positive values were considered effective, while significant negative values were considered counterproductive. Data were analyzed using the MIXED procedure of SAS (SAS®, 2001). Statistical significance was declared at $P < 0.05$.

RESULTS & DISCUSSION

NovaSilplus, Toxynil+, Astra Ben 20A, and Milbond-TX significantly reduced milk aflatoxin concentration, secretion, and transfer (Table 1.). Other treatment products did not reduce AFM$_1$ concentration, secretion or aflatoxin transfer from feed to milk. The data suggest that regardless of which parameter measured, milk yield and aflatoxin intake typically have little effect on the results. It can be concluded that determining milk aflatoxin concentration is appropriate for estimating dietary aflatoxin exposure and for evaluating adsorbent product effectiveness.

Of the treatment products used in this experiment, NovaSil, Astra Ben 20 and MTB-100 have been evaluated *in vivo* for potential to reduce milk aflatoxin concentrations. In previous work, Astra Ben 20 fed at 1.2% and MTB-100 fed at 0.05% of diet DM were both effective at reducing milk AFM_1 concentrations (Diaz et al., 2004). NovaSil fed at 0.5% or 1.0% of diet DM reduced milk AFM_1 concentrations (Harvey et al., 1991). Therefore, this work confirms the previous responses of Astra Ben 20 and NovaSil, but not that of MTB-100.

Kannewischer et al. (2005) conducted an *in vitro* experiment in which each product in the current study, as well as others, were characterized based on ammonium acetate exchangeable cations ($cmol_c$/kg) and organic carbon (%). This type of product characterization does not necessarily explain the results seen in either study. The method of determining the physical properties of adsorbent products is not yet proven to be a reliable means to predict product effectiveness. It is likely that the structure of the adsorbents may play an important role in the ability of a product to bind aflatoxin. For example, bentonites, or montmorillonites, have greater binding when compared to zeolites due to their ability to bind both on the surface and in the interlayer region of the molecule (Tomasevic-Canovic et al., 2001).

The differences in estimates of AFB_1 binding *in vivo* vs. *in vitro* have been previously noted by Scheideler (1993), Dwyer et al. (1997), and Diaz et al. (2004). Each treatment product was also evaluated *in vitro* using an AFB_1 binding assay. Percent bound aflatoxin for each treatment product is reported in Table 2. All products bound greater than 96% with the exception of MTB-100, which bound 43.4%. This value for Astra Ben 20A was similar to previously reported data from this laboratory, but for MTB-100 was considerably lower (43.43% vs. 96.6%) (Diaz et al., 2002).

Milk production and DMI were measured on days one and two, when there was no aflatoxin in the feed, and on days 5 and 6 of each replicate, when cows were receiving an aflatoxin-contaminated diet. Aflatoxin intake significantly reduced DMI (24.0 kg vs. 22.5 kg; $P = 0.001$), but had no effect on milk production during this time period ($P = 0.22$). Production may respond to aflatoxin over a longer duration of feeding (Applebaum et al., 1982). There were no significant effects of treatment additives on milk production ($P = 0.636$) or DMI ($P = 0.593$).

CONCLUSIONS

The success of NovaSilplus, Toxynil+, Astra Ben 20A, and Milbond-TX in reducing AFM_1 concentration of milk of dairy cows suggests that these adsorbents have the potential as feed additives to prevent aflatoxin contamination of milk. The addition of aflatoxin adsorbent products to dairy feed may be useful in warm, humid climatic conditions that are most favorable for aflatoxin formation. Aflatoxin adsorbents may have a role in managing the risk of contaminating milk with aflatoxin and the resulting economic loss. The low cost of adsorbent products would encourage their use. The FDA has not yet approved any products for the claim of adsorption of aflatoxin.

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REFERENCES

Applebaum, R.S., R.E. Brackett, D.W. Wiseman, and E.H. Marth. 1982. Responses of dairy cows to dietary aflatoxin: feed intake and yield, toxin content, and quality of milk of cows treated with pure and impure aflatoxin. J. Dairy Sci. 65:1503-1508

Diaz, D.E., W.M. Hagler Jr., B.A. Hopkins, and L.W. Whitlow. 2002. Aflatoxin binders I: in vitro binding assay for aflatoxin B1 by several potential sequestering agents. Mycopathologia. 156(3):223-226.

Diaz, D.E., W.M. Hagler Jr., J.T. Blackwelder, J.A. Eve, B.A. Hopkins, K.L. Anderson, F.T. Jones, and L.W. Whitlow. 2004. Aflatoxin binders II: reduction of aflatoxin M1 in milk by sequestering agents of cows consuming aflatoxin in feed. Mycopathologia. 157(2):233-241.

Dwyer, M. R., L.F. Kubena, R.B. Harvey, K. Mayura, A.B. Sarr, S. Buckley, R. H. Bailey, and T.D. Phillips. 1997. Effects of inorganic adsorbents and cyclopiazonic acid in brioler chicks. Poult. Sci. 76:1141-1149.

Eaton, D.L., H.S. Ramsdell, and G.E. Neal. 1994. Biotransformation of Aflatoxins. Pages 45-72 in The Toxicology of Aflatoxins. D.L. Eaton and J.D. Groopman, eds. Academic Press, Inc. San Diego, CA.

Kannewischer, I., M.G. Tenorio Arvide, G.N. White, and J.B. Dixon. 2005. Clay Science of Japan. 12(Suppl. 2). Intl. Clay Conf. Japan.

Lemke, S.L., S.E. Ottinger, K. Mayura, C.L. Ake, K. Pimpukdee, N. Wang, and T.D. Phillips. 2001. Development of a multi-tiered approach to the in vitro prescreening of clay-based enterosorbents. Anim. Feed Sci. Tech. 93:17-29.

Maryamma, K.I., A. Rajan, B. Gangadharan, and C.B. Manomohan. 1991. In vitro and in vivo studies on aflatoxin B_1 neutralization. Ind. J. Anim. Sci. 61:58-60.

National Research Council. 2001. Nutrient requirements of dairy cattle. 7th ed. Natl. Acad. Sci., Washington, DC.

SAS. 2001. SAS/STAT User's Guide: Statistics, Version 8.2, SAS Institute Inc., Cary, NC

Scheideler, S. E. 1993. Effects of various types of aluminosilicates and aflatoxin B1 on aflatoxin toxicity, chick performance, and mineral status. Poult. Sci. 72: 282-288.

Tomasevic-Canovic, M., A. Dakovic, V. Markovic, and D. Stojsic. 2001. The effect of exchangeable cations in clinoptilolite and montmorillonite on the adsorption of aflatoxin B_1. J. Serb. Chem. Soc. 66(5):555-561.

Van Egmond, H.P. and W.E. Paulsch. 1986. Mycotoxins in milk and milk products. Neth. Milk Dairy J. 40:175-188.

Table 1. Percent reductions in milk aflatoxin concentration, milk aflatoxin secretion, and milk aflatoxin transfer due to the addition of adsorbent products at 0.5% diet DM.

	% Reduction		
Treatment Identification	Milk Aflatoxin Concentration	Milk Aflatoxin Secretion	Aflatoxin Transfer from feed to milk
MTB-100	-7.81	-6.71	-3.60
UltraSorb	7.36	7.85	7.59
Mexsil	6.62	8.00	7.19
NovaSilplus	40.39*	42.59*	42.09*
Toxynil+	34.98*	36.36*	34.45*
Condition Ade	7.85	13.79	13.23
Astra Ben 20A	48.90*	52.28*	48.44*
Milbond-TX	46.49*	48.46*	44.55*
Pooled Standard Error	12.69	13.75	13.12

*Values are different from zero when $P < 0.05$.

Table 2. Percent aflatoxin bound by one of eight adsorbent products *in vitro*[1].

Treatment Identification	%	Coefficient of Variation
MTB-100	43.43	2.28
UltraSorb	98.80	1.83
Mexsil	97.48	1.28
NovaSilplus	99.45	4.00
Toxynil+	99.31	6.91
Condition Ade	99.51	8.97
Astra Ben 20A	96.17	4.89
Milbond-TX	98.21	2.10

[1] *In vitro* binding method described by Diaz et al., 2004.

APPENDIX

Table 3. Aflatoxin concentrations in feed and milk reported in previous studies.

References	Form of AF Measured*	Aflatoxin Concentration in Feed	Aflatoxin Concentration in Milk
Allcroft & Roberts, 1968	AFB$_1$	50	0.33
		100	0.66
		150.28	1.22
		285.71	3
		857.14	7.9
		1142.86	9.5
		1400	13.3
		51.43	0.33
Applebaum et al., 1982	AFB$_1$	461	2.74
		515	3.78
		429	2.16
		430	2.49
		510	2.1
		550	4.4
		770	9.22
		425	1.05
		680	10.58
		920	1.35
Diaz et al., 2004	AFB$_1$	55	1.24
Fremy & Quillardet, 1985	AFB$_1$	54.29	0.30
		54.29	0.11
Frobish et al., 1986	AFB$_1$	28.11	0.36
		19.94	0.33
		24.06	0.35
		65.37	0.74
		45.09	0.51
		55.26	0.63
		142.34	1.5
		94.57	1.72
		118.46	1.61
		55.2	0.62
		42.4	0.59
		48.8	0.61
		63.77	0.51
		50.57	0.55
		57.2	0.53
Galvano et al., 1996	AFB$_1$	3.22	0.68
		3.22	0.70
		3.22	0.70
		3.84	0.76
		3.84	0.78
		3.84	0.81
Harvey et al., 1991	AF	200	1.85
		200	1.99
		100	0.91
		100	0.9

References	Form of AF Measured*	Aflatoxin Concentration in Feed	Aflatoxin Concentration in Milk
Munksgaard et al., 1987	AFB_1	3.26	0.04
		3.26	0.05
		3.26	0.04
		8.11	0.06
		8.11	0.12
		8.11	0.08
		12.91	0.07
		12.91	0.11
		12.91	0.25
		17.77	0.11
		17.77	0.12
		17.77	0.11
Patterson et al., 1980	AFB_1	9.89	0.16
		8.86	0.19
		10.2	0.21
Polan et al., 1974	AFB_1	3	0
		16	0.01
		86	0.26
		466	0.82
Sassahara et al., 2005	AFB_1	2	0.33
		7.26	1.58
		2	0.80
		29.04	1.97
		7.26	0.31
		2	0.40
		2	0.30
		2	0.41
		2	0.40
		2	0.34
Shreeve et al., 1979	AFB_1	20	0.06
Stubblefield et al., 1983	AFB_1	350	16.35
		350	16.55
Veldman, 1992	AFB_1	2	0.01
		10	0.08
		0.90	0.01
		4.47	0.09
		2.05	0.04

*AF = aflatoxin; AFB_1 = aflatoxin B_1

Table 4. AFM$_1$ (ppb) concentration of milk from cows consuming approximately 170 ppb aflatoxin for day 1; no aflatoxin or additive in the diet, day 5 (rep1) and 10 (rep 2) when cows were consuming aflatoxin-contaminated diets without and additive; days 11, 12 (rep 1) and days 21, 22 (rep 2) when cows were consuming aflatoxin-contaminated diets with inclusion of an additive, except for control that received no additive.

Cow No.	Date	Day	AFM$_1$, ppb
1571	11/1-11/2	1	0
1585	11/1-11/2	1	0
1619	11/1-11/2	1	0.017
1624	11/1-11/2	1	0.021
1675	11/1-11/2	1	0
1681	11/1-11/2	1	0
1686	11/1-11/2	1	0
1708	11/1-11/2	1	0
1723	11/1-11/2	1	0
1728	11/1-11/2	1	0.028
1732	11/1-11/2	1	0.018
1735	11/1-11/2	1	0.019
1736	11/1-11/2	1	0
1747	11/1-11/2	1	0.016
1749	11/1-11/2	1	0
1756	11/1-11/2	1	0.016
1762	11/1-11/2	1	0
1763	11/1-11/2	1	0
1784	11/1-11/2	1	0
1785	11/1-11/2	1	0
1793	11/1-11/2	1	0
1794	11/1-11/2	1	0
1807	11/1-11/2	1	0.032
1808	11/1-11/2	1	0.024
1818	11/1-11/2	1	0
1819	11/1-11/2	1	0
1820	11/1-11/2	1	0.023
1830	11/1-11/2	1	0
1839	11/1-11/2	1	0
1842	11/1-11/2	1	0.029
1843	11/1-11/2	1	0
1844	11/1-11/2	1	0
1846	11/1-11/2	1	0.023
1850	11/1-11/2	1	0
1852	11/1-11/2	1	0
1855	11/1-11/2	1	0
1856	11/1-11/2	1	0.015
1857	11/1-11/2	1	0
1858	11/1-11/2	1	0.017
1860	11/1-11/2	1	0
1864	11/1-11/2	1	0
1867	11/1-11/2	1	0
1868	11/1-11/2	1	0.014
1869	11/1-11/2	1	0
1871	11/1-11/2	1	0.012

Cow No.	Date	Day	AFM_1, ppb
1873	11/1-11/2	1	0
1875	11/1-11/2	1	0.021
1877	11/1-11/2	1	0
1878	11/1-11/2	1	0
1879	11/1-11/2	1	0
1881	11/1-11/2	1	0
1886	11/1-11/2	1	0
1890	11/1-11/2	1	0.016
1892	11/1-11/2	1	0.015
1895	11/1-11/2	1	0
1896	11/1-11/2	1	0.017
1897	11/1-11/2	1	0
1898	11/1-11/2	1	0
1899	11/1-11/2	1	0
1901	11/1-11/2	1	0
1571	11/5-11/6	5	2.03
1585	11/5-11/6	5	1.89
1619	11/5-11/6	5	2.428
1624	11/5-11/6	5	3.268
1675	11/5-11/6	5	3.338
1681	11/5-11/6	5	2.392
1686	11/5-11/6	5	2.224
1708	11/5-11/6	5	2.17
1723	11/5-11/6	5	2.952
1728	11/5-11/6	5	6.432
1732	11/5-11/6	5	2.372
1735	11/5-11/6	5	5.832
1736	11/5-11/6	5	2.044
1747	11/5-11/6	5	2.598
1749	11/5-11/6	5	2.51
1756	11/5-11/6	5	1.806
1762	11/5-11/6	5	5.04
1763	11/5-11/6	5	2.646
1784	11/5-11/6	5	6.172
1784	11/5-11/6	5	4.428
1785	11/5-11/6	5	2.918
1793	11/5-11/6	5	3.558
1794	11/5-11/6	5	2.608
1807	11/5-11/6	5	1.559
1808	11/5-11/6	5	1.563
1818	11/5-11/6	5	3.374
1819	11/5-11/6	5	3.346
1820	11/5-11/6	5	3.244
1830	11/5-11/6	5	1.164
1839	11/5-11/6	5	3.104
1842	11/5-11/6	5	2.272
1843	11/5-11/6	5	2.934
1844	11/5-11/6	5	2.612
1846	11/5-11/6	5	3.62

Cow No.	Date	Day	AFM$_1$, ppb
1850	11/5-11/6	5	1.289
1852	11/5-11/6	5	2.484
1855	11/5-11/6	5	1.795
1856	11/5-11/6	5	2.536
1857	11/5-11/6	5	1.597
1858	11/5-11/6	5	4.936
1860	11/5-11/6	5	3.19
1864	11/5-11/6	5	2.616
1867	11/5-11/6	5	5.628
1868	11/5-11/6	5	2.326
1869	11/5-11/6	5	3.772
1871	11/5-11/6	5	2.796
1873	11/5-11/6	5	3.908
1875	11/5-11/6	5	3.892
1877	11/5-11/6	5	3.728
1878	11/5-11/6	5	1.672
1879	11/5-11/6	5	2.556
1881	11/5-11/6	5	3.656
1886	11/5-11/6	5	3.404
1890	11/5-11/6	5	1.634
1892	11/5-11/6	5	4.884
1895	11/5-11/6	5	3.632
1896	11/5-11/6	5	1.834
1897	11/5-11/6	5	3.012
1898	11/5-11/6	5	2.966
1899	11/5-11/6	5	2.412
1901	11/5-11/6	5	4.008
1571	11/10-11/11	10	1.741
1585	11/10-11/11	10	1.057
1619	11/10-11/11	10	1.565
1624	11/10-11/11	10	5.252
1675	11/10-11/11	10	1.391
1681	11/10-11/11	10	3.638
1686	11/10-11/11	10	0.995
1708	11/10-11/11	10	2.736
1723	11/10-11/11	10	1.738
1728	11/10-11/11	10	2.714
1732	11/10-11/11	10	0.767
1735	11/10-11/11	10	4.008
1736	11/10-11/11	10	2.308
1747	11/10-11/11	10	1.869
1749	11/10-11/11	10	1.04
1756	11/10-11/11	10	1.592
1762	11/10-11/11	10	4.42
1763	11/10-11/11	10	1.224
1784	11/10-11/11	10	3.78
1785	11/10-11/11	10	2.456
1793	11/10-11/11	10	1.515
1794	11/10-11/11	10	2.092

Cow No.	Date	Day	AFM$_1$, ppb
1807	11/10-11/11	10	2.726
1808	11/10-11/11	10	1.754
1818	11/10-11/11	10	1.493
1819	11/10-11/11	10	1.411
1820	11/10-11/11	10	2.588
1830	11/10-11/11	10	1.828
1839	11/10-11/11	10	1.775
1842	11/10-11/11	10	2.268
1843	11/10-11/11	10	3.452
1844	11/10-11/11	10	2.424
1846	11/10-11/11	10	1.425
1850	11/10-11/11	10	4.132
1852	11/10-11/11	10	0.865
1855	11/10-11/11	10	4.204
1856	11/10-11/11	10	2.664
1857	11/10-11/11	10	0.86
1858	11/10-11/11	10	2.612
1860	11/10-11/11	10	2.82
1864	11/10-11/11	10	0.869
1867	11/10-11/11	10	1.723
1868	11/10-11/11	10	1.559
1869	11/10-11/11	10	2.318
1871	11/10-11/11	10	1.344
1873	11/10-11/11	10	2.013
1875	11/10-11/11	10	3.808
1877	11/10-11/11	10	3.018
1878	11/10-11/11	10	3.844
1879	11/10-11/11	10	2.68
1881	11/10-11/11	10	1.553
1886	11/10-11/11	10	3.044
1890	11/10-11/11	10	1.184
1892	11/10-11/11	10	4.684
1895	11/10-11/11	10	2.624
1896	11/10-11/11	10	1.346
1897	11/10-11/11	10	2.09
1898	11/10-11/11	10	3.048
1899	11/10-11/11	10	1.457
1901	11/10-11/11	10	1.756
1571	11/11-11/12	11	0.992
1585	11/11-11/12	11	1.449
1619	11/11-11/12	11	0.812
1624	11/11-11/12	11	5.376
1675	11/11-11/12	11	1.427
1681	11/11-11/12	11	3.424
1686	11/11-11/12	11	1.055
1708	11/11-11/12	11	1.195
1723	11/11-11/12	11	2.496
1728	11/11-11/12	11	1.915
1732	11/11-11/12	11	1.022

Cow No.	Date	Day	AFM_1, ppb
1735	11/11-11/12	11	2.958
1736	11/11-11/12	11	3.134
1747	11/11-11/12	11	1.099
1749	11/11-11/12	11	0.65
1756	11/11-11/12	11	1.196
1762	11/11-11/12	11	3.34
1763	11/11-11/12	11	1.248
1784	11/11-11/12	11	2.528
1785	11/11-11/12	11	1.33
1793	11/11-11/12	11	1.418
1794	11/11-11/12	11	3.05
1807	11/11-11/12	11	2.102
1808	11/11-11/12	11	1.29
1818	11/11-11/12	11	1.229
1819	11/11-11/12	11	2.344
1820	11/11-11/12	11	2.396
1830	11/11-11/12	11	3.348
1839	11/11-11/12	11	2.168
1842	11/11-11/12	11	2.652
1843	11/11-11/12	11	4.516
1844	11/11-11/12	11	1.498
1846	11/11-11/12	11	2.72
1850	11/11-11/12	11	3.534
1852	11/11-11/12	11	0.578
1855	11/11-11/12	11	2.786
1856	11/11-11/12	11	3.724
1857	11/11-11/12	11	0.564
1858	11/11-11/12	11	3.61
1860	11/11-11/12	11	2.107
1864	11/11-11/12	11	1.001
1867	11/11-11/12	11	1.865
1868	11/11-11/12	11	1.641
1869	11/11-11/12	11	2.322
1871	11/11-11/12	11	1.307
1873	11/11-11/12	11	2.092
1875	11/11-11/12	11	4.244
1877	11/11-11/12	11	4.24
1878	11/11-11/12	11	2.842
1879	11/11-11/12	11	2.792
1881	11/11-11/12	11	1.305
1886	11/11-11/12	11	3.082
1890	11/11-11/12	11	1.47
1892	11/11-11/12	11	3.66
1895	11/11-11/12	11	2.318
1896	11/11-11/12	11	1.569
1897	11/11-11/12	11	1.199
1898	11/11-11/12	11	2.92
1899	11/11-11/12	11	1.219
1901	11/11-11/12	11	1.473

Cow No.	Date	Day	AFM$_1$, ppb
1571	11/15-11/16	15	3.09
1585	11/15-11/16	15	1.548
1619	11/15-11/16	15	2.074
1624	11/15-11/16	15	5.136
1675	11/15-11/16	15	3.418
1681	11/15-11/16	15	1.801
1686	11/15-11/16	15	0.651
1708	11/15-11/16	15	1.335
1723	11/15-11/16	15	4.472
1728	11/15-11/16	15	5.072
1732	11/15-11/16	15	1.119
1735	11/15-11/16	15	2.024
1736	11/15-11/16	15	2.812
1747	11/15-11/16	15	2.442
1749	11/15-11/16	15	1.33
1756	11/15-11/16	15	2.192
1762	11/15-11/16	15	2.94
1763	11/15-11/16	15	1.785
1785	11/15-11/16	15	4.332
1793	11/15-11/16	15	1.429
1794	11/15-11/16	15	2.182
1807	11/15-11/16	15	4.3
1808	11/15-11/16	15	2.544
1818	11/15-11/16	15	4.396
1819	11/15-11/16	15	1.008
1820	11/15-11/16	15	3.136
1830	11/15-11/16	15	1.722
1839	11/15-11/16	15	0.952
1842	11/15-11/16	15	3.04
1843	11/15-11/16	15	3.088
1844	11/15-11/16	15	1.669
1846	11/15-11/16	15	3.8
1850	11/15-11/16	15	2.834
1852	11/15-11/16	15	2.036
1855	11/15-11/16	15	1.844
1856	11/15-11/16	15	2.082
1857	11/15-11/16	15	3.47
1858	11/15-11/16	15	4.036
1860	11/15-11/16	15	3.634
1864	11/15-11/16	15	3.046
1867	11/15-11/16	15	4.984
1868	11/15-11/16	15	2.714
1869	11/15-11/16	15	3.564
1871	11/15-11/16	15	1.606
1873	11/15-11/16	15	3.97
1875	11/15-11/16	15	4.816
1877	11/15-11/16	15	1.625
1878	11/15-11/16	15	3.056
1879	11/15-11/16	15	1.816

Cow No.	Date	Day	AFM$_1$, ppb
1881	11/15-11/16	15	1.889
1886	11/15-11/16	15	2.47
1890	11/15-11/16	15	3.052
1892	11/15-11/16	15	4.428
1895	11/15-11/16	15	0.968
1896	11/15-11/16	15	1.983
1897	11/15-11/16	15	2.051
1898	11/15-11/16	15	3.472
1899	11/15-11/16	15	2.632
1901	11/15-11/16	15	2.476
1571	11/20-11/21	20	0.457
1585	11/20-11/21	20	0.966
1619	11/20-11/21	20	1.152
1624	11/20-11/21	20	1.136
1675	11/20-11/21	20	1.803
1681	11/20-11/21	20	0.406
1686	11/20-11/21	20	0.67
1708	11/20-11/21	20	0.801
1723	11/20-11/21	20	1.025
1728	11/20-11/21	20	1.301
1732	11/20-11/21	20	0.618
1735	11/20-11/21	20	1.676
1736	11/20-11/21	20	0.3
1747	11/20-11/21	20	0.422
1749	11/20-11/21	20	1.071
1756	11/20-11/21	20	1.001
1762	11/20-11/21	20	1.232
1763	11/20-11/21	20	1.295
1784	11/20-11/21	20	1.495
1785	11/20-11/21	20	1.628
1793	11/20-11/21	20	0.4
1794	11/20-11/21	20	0.716
1807	11/20-11/21	20	1.733
1808	11/20-11/21	20	0.443
1818	11/20-11/21	20	2.009
1819	11/20-11/21	20	1.114
1820	11/20-11/21	20	0.871
1830	11/20-11/21	20	1.115
1839	11/20-11/21	20	0.392
1842	11/20-11/21	20	1.173
1843	11/20-11/21	20	0.998
1844	11/20-11/21	20	0.844
1846	11/20-11/21	20	1.091
1850	11/20-11/21	20	1.123
1852	11/20-11/21	20	1.248
1855	11/20-11/21	20	0.867
1856	11/20-11/21	20	0.679
1857	11/20-11/21	20	1.166
1858	11/20-11/21	20	1.215

Cow No.	Date	Day	AFM$_1$, ppb
1860	11/20-11/21	20	1.401
1864	11/20-11/21	20	1.274
1867	11/20-11/21	20	0.952
1868	11/20-11/21	20	0.906
1869	11/20-11/21	20	0.559
1871	11/20-11/21	20	1.371
1873	11/20-11/21	20	1.05
1875	11/20-11/21	20	1.687
1877	11/20-11/21	20	1.04
1878	11/20-11/21	20	1.598
1879	11/20-11/21	20	0.726
1881	11/20-11/21	20	1.452
1886	11/20-11/21	20	1.835
1890	11/20-11/21	20	0.996
1892	11/20-11/21	20	0.519
1895	11/20-11/21	20	0.615
1896	11/20-11/21	20	0.412
1897	11/20-11/21	20	0.632
1898	11/20-11/21	20	0.6
1899	11/20-11/21	20	0.509
1901	11/20-11/21	20	1.205
1571	11/21-11/22	21	0.871
1585	11/21-11/22	21	0.586
1619	11/21-11/22	21	1.508
1624	11/21-11/22	21	1.777
1675	11/21-11/22	21	2.012
1681	11/21-11/22	21	0.562
1686	11/21-11/22	21	0.516
1708	11/21-11/22	21	0.414
1723	11/21-11/22	21	0.426
1728	11/21-11/22	21	2.458
1732	11/21-11/22	21	0.936
1735	11/21-11/22	21	2.216
1736	11/21-11/22	21	0.595
1747	11/21-11/22	21	0.452
1749	11/21-11/22	21	1.293
1756	11/21-11/22	21	1.187
1762	11/21-11/22	21	1.651
1763	11/21-11/22	21	1.258
1784	11/21-11/22	21	1.299
1785	11/21-11/22	21	0.784
1793	11/21-11/22	21	0.525
1794	11/21-11/22	21	0.728
1807	11/21-11/22	21	1.723
1808	11/21-11/22	21	0.675
1818	11/21-11/22	21	2.212
1819	11/21-11/22	21	1.428
1820	11/21-11/22	21	1.322
1830	11/21-11/22	21	1.018

Cow No.	Date	Day	AFM_1, ppb
1839	11/21-11/22	21	0.484
1842	11/21-11/22	21	2.043
1843	11/21-11/22	21	0.873
1844	11/21-11/22	21	0.564
1846	11/21-11/22	21	1.352
1850	11/21-11/22	21	1.314
1852	11/21-11/22	21	1.256
1855	11/21-11/22	21	0.893
1856	11/21-11/22	21	0.583
1857	11/21-11/22	21	1.129
1858	11/21-11/22	21	1.502
1860	11/21-11/22	21	1.198
1864	11/21-11/22	21	1.101
1867	11/21-11/22	21	0.948
1868	11/21-11/22	21	0.804
1869	11/21-11/22	21	0.535
1871	11/21-11/22	21	1.435
1873	11/21-11/22	21	1.23
1875	11/21-11/22	21	0.453
1877	11/21-11/22	21	1.03
1878	11/21-11/22	21	1.385
1879	11/21-11/22	21	1.102
1881	11/21-11/22	21	1.819
1886	11/21-11/22	21	2.322
1890	11/21-11/22	21	1.294
1892	11/21-11/22	21	0.456
1895	11/21-11/22	21	0.337
1896	11/21-11/22	21	0.236
1897	11/21-11/22	21	0.769
1898	11/21-11/22	21	0.617
1899	11/21-11/22	21	0.457
1901	11/21-11/22	21	0.634

Table 5. Ingredient composition of total mixed ration fed to all cows.

Ingredient	% of Dry Matter
Corn Silage	44.8
Cottonseed	8.9
Corn	20.7
48% Soybean Meal	17.4
Cottonseed Hulls	5.2
Lime	1.1
Dicalcium phosphate	0.57
Salt	0.38
Bicarbonate	0.57
Trace Mineral-Vitamin	0.19
Dynamate[1]	0.19

[1] Pitman-Moore Inc.(Chicago, IL); contained 22% S, 18% K, and 11 % Mg.