562 RUNNING HEAD: POTENTIAL ADSORBENTS OF AFLATOXIN
563
564 **The Effect of Feed Additives on Aflatoxin in Milk of Dairy Cows Fed Aflatoxin-**
565 **contaminated Diets.**
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590 **ABSTRACT**

591 Sixty lactating Holstein cows were used in a replicated block experiment to
592 determine the efficacy of eight feed additives to reduce the transfer of aflatoxin (**AF**)
593 from feed to milk. In each replicate, six cows were allocated to each treatment group and
594 12 to a control group. All cows were fed the same aflatoxin-contaminated total mixed
595 ration (**TMR**) for 11 d with either no additive (control) or one of eight additives included
596 at 0.5% of the TMR dry matter (**DM**) during the last 6 d of the replicate. Milk samples
597 were collected when additives were fed or not fed to evaluate changes in milk AF
598 concentration, milk AF secretion (milk AF concentration × milk yield); and AF transfer
599 from feed to milk (AF secretion as a percentage of AF intake). All changes were
600 normalized to the control group and expressed such that a positive percentage indicated a
601 reduction in AF values associated with feed additive inclusion. Four of the eight
602 additives resulted in significant reductions ($P < 0.05$) ranging from 34.98 to 48.9% for
603 milk AF concentration, 36.36 to 52.28% for milk AF secretion, and 34.45 to 48.44% for
604 AF transfer. Dry matter intake (**DMI**) was significantly reduced ($P < 0.001$) by the
605 consumption of AF, while milk production was not affected during the same time period.
606 Neither DMI nor milk production were affected by the addition of treatment products to
607 the diet when compared to control ($P > 0.05$).

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

609

610 **INTRODUCTION**

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612 Feeding aflatoxin-contaminated diets to lactating animals results in secretion of
613 aflatoxin M_1 (**AFM_1**) in the milk (van Egmond and Paulsch, 1986). AFM_1 is the
614 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

640 treatment diet was blended using a DataRanger (American Calan Inc., Northwood, NH)
641 mixer and fed to cows through Calan (American Calan Inc., Northwood, NH) feeding
642 stations. After each treatment ration was fed, the mixer was flushed with 28 kg silage to
643 avoid cross contamination of treatment products. Cows consumed 170 ppb total aflatoxin
644 which was calculated based on the milk aflatoxin concentration of the control cows and
645 assuming 1.7% transfer of aflatoxin from feed to milk (Frobish et al., 1986).

646 ***Experimental Design***

647 Sixty lactating Holstein cows from the Piedmont Research Station in Salisbury,
648 NC were randomly assigned to nine treatment groups. The treatment groups were 1)
649 MTB-100 (Alltech, Inc., Nicholasville, KY); 2) UltraSorb (Micron Bio-systems, Inc.,
650 Buena Vista, VA); 3) Mexsil (Karluis Enterprises, Queretaro, Mexico); 4) NovaSilplus
651 (Trouw Nutrition, Highland, IL); 5) Toxynil+ (INVE Technologies, Dendermonde,
652 Belgium); 6) Condition Ade (Oil·Dri Corporation, Chicago, IL); 7) Astra Ben 20A
653 (Prince Agri Products, Inc., Quincy, IL); 8) Milbond-TX (Milwhite, Inc., Brownsville,
654 TX); and 9) Control (no additive). MTB-100, UltraSorb, and Toxynil+ were composed
655 of yeast and silicates, while the remaining adsorbents were composed of silicates only.
656 The adsorbents were added to the diet at 0.5% of the diet dry matter or approximately
657 100g/cow/day. The experiment was designed as a replicated block with six cows
658 allocated to each treatment group and twelve cows in the control group, blocked by both
659 milk production and parity (1or >1). In replicate two, cows were reallocated so that no
660 cow remained on the same treatment and remained blocked by parity and milk
661 production. The lower producers of the herd were used for the experiment, with an
662 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.

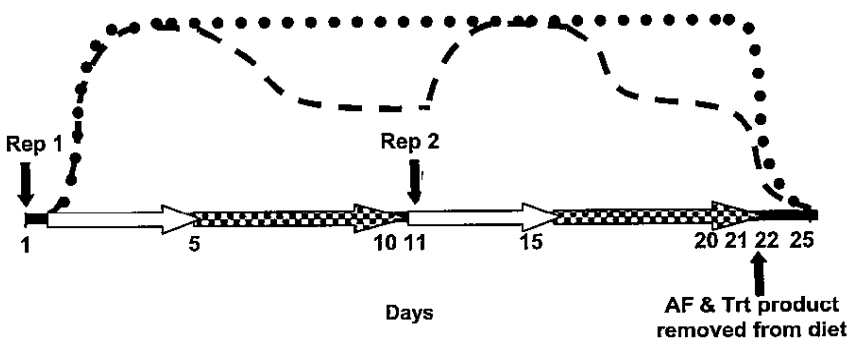

Figure 1. Timeline illustrating the two replicates of the experiment, periods of AF intake ☐, AF plus treatment product ▩, milk sampling days, the theoretical AFM_1 concentration in response to feeding AF ..., and the theoretical concentration following the addition of an adsorbent product to an AF-contaminated diet ---.

685 ***AFM_1 Analysis***

686 Milk samples were analyzed for AFM1 using HPLC and VICAM affinity
687 columns as described by Viacom L.P. Instruction Manual # 6M-MC 9512-3 (Viacom
688 L.P., Watertown, MA). Milk samples were composited by day then defatted prior to
689 analysis. A 35 ml sample of defatted milk was then passed through the AFM_1 affinity
690 column and filtered. Water was passed through the column and AFM_1 eluted and
691 collected in a scintillation vial. The eluate was analyzed by HPLC. Concentrations of
692 AFM_1 were determined relative to a quantitative AFM_1 standard.

693 ***Statistical Analysis***

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

703 **RESULTS & DISCUSSION**

704 NovaSilplus, Toxynil+, Astra Ben 20A, and Milbond-TX significantly reduced
705 milk aflatoxin concentration, secretion, and transfer (Table 1.). Other treatment products
706 did not reduce AFM_1 concentration, secretion or aflatoxin transfer from feed to milk.
707 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

731 bound aflatoxin for each treatment product is reported in Table 2. All products bound
732 greater than 96% with the exception of MTB-100, which bound 43.4%. This value for
733 Astra Ben 20A was similar to previously reported data from this laboratory, but for
734 MTB-100 was considerably lower (43.43% vs. 96.6%) (Diaz et al., 2002).

735 Milk production and DMI were measured on days one and two, when there was
736 no aflatoxin in the feed, and on days 5 and 6 of each replicate, when cows were receiving
737 an aflatoxin-contaminated diet. Aflatoxin intake significantly reduced DMI (24.0 kg vs.
738 22.5 kg; $P = 0.001$), but had no effect on milk production during this time period ($P =$
739 0.22). Production may respond to aflatoxin over a longer duration of feeding
740 (Applebaum et al., 1982). There were no significant effects of treatment additives on
741 milk production ($P = 0.636$) or DMI ($P = 0.593$).

742 **CONCLUSIONS**

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

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841 Table 1. Percent reductions in milk aflatoxin concentration, milk aflatoxin secretion,
842 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

843 *Values are different from zero when $P < 0.05$.
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871 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

872 [1]*In vitro* binding method described by Diaz et al., 2004.

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