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Reduction of Carryover of Aflatoxin from Cow Feed to Milk by Addition of Activated Carbons

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ABSTRACT

According to a double-reversal experimental design on 12 late-lactation Friesian cows the effect of two activated carbons (ACs) (CAC1 and CAC2) and a hydrated sodium calcium aluminosilicate (HSCAS) on carryover of aflatoxin B_1 (AFB_1) from feed to aflatoxin M_1 (AFM_1) in milk was determined. Cows were fed a basal diet containing AFB_1 naturally contaminated corn meal and copra. During week 1 cows were fed diets containing AFB_1 alone (11.28 μg of AFB_1/kg of feed); in week 2 the diets contained AFB_1 plus 2.0% sorbent; and in week 3 the diets again contained AFB_1 alone (13.43 μg of AFB_1/kg of feed). ACs reduced the analytical content of AFB_1 in the pelleted feed by from 40.6% to 73.6%, whereas reduction by HSCAS was 59.2%. The AFM_1 concentrations in milk in weeks 1 and 3 were higher than that in week 2. Decreases in the AFM_1 excreted in the milk by addition to feed of 2% of the sorbents ranged from 22% to 45%. CAC1 and HSCAS were significantly different from each other in reducing the AFM_1 concentration in milk (45.3% versus 32.5%); these reductions were significantly higher than that of CAC2 (22.0%). Carryover reduction by addition of CAC1 (50%) was significantly higher than that of HSCAS (36%). Addition of 2% CAC2 did not allow pelleting of feed because of the caking action of this carbon. The lower performance of CAC2 could be related to the unsuccessful pelleting. The addition of ACs did not influence feed intake, milk production, milk composition, or body weight. Our results suggest that ACs, high-affinity sorbents for AFB_1 in vitro, are efficacious in reducing AFB_1 carryover from cow feed to milk. Further in vivo investigations should establish lower amounts of ACs which can be efficacious.

Key words: Aflatoxins, milk, activated carbons, carryover, feed

Aflatoxins (AF) are a group of highly toxic secondary metabolic products of *Aspergillus flavus* and *Aspergillus parasiticus* that easily occur on feeds and foods. As AF are carcinogenic, teratogenic, and mutagenic to animals, including humans, detoxification of aflatoxin-contaminated foods and feeds is one of the most serious problems of food hygiene.

Dairy cows fed aflatoxin B_1 (AFB_1)-contaminated diets secrete into milk amounts of the main hepatic AFB_1 metabolite, aflatoxin M_1 (AFM_1), as a function of AFB_1 intake and lactation stage *(33)*. AFM_1 toxicity is similar to that of the original molecule as shown by trials carried out on ducks *(25)*, rats *(23)*, and trout *(27)*, whereas its mutagenic potential seems to be lower *(1)*. Recently AFB_1 and AFM_1 were categorized as class 1 and 2B (or probable) human carcinogens, respectively *(26)*. As milk is one of the most important human foods and the main nutrient for growing young, who are notably vulnerable and potentially more sensitive than adults, numerous methods of detoxification have been tested *(22)*.

The addition of sorbents to contaminated feeds seems to be the most efficacious strategy for preventing aflatoxicosis. Sorbents act as inabsorbable carriers that adsorb toxic molecules, thereby preventing or reducing their absorption in the intestinal tract. Some in vitro tests *(20)* showed that hydrated sodium calcium aluminosilicates (HSCASs) were particularly efficacious in binding aflatoxin. Harvey et al. *(10)* conducted a detoxification test by adding HSCAS to dairy cow feeds having 200 μg of AFB_1/kg and obtained a significant reduction in carryover, also confirming the efficacy of the treatment even at the low contamination levels frequently found in practice.

Pietri et al. *(21)* tested the effectiveness of a zeolite in reducing carryover of AFB_1 and did not detect any effect on carryover. Veldman *(32)* revealed a 33% and 0% carryover reduction by bentonite and HSCAS, respectively, and detected the formation of an inert, stable complex between bentonite and AFB_1 capable of preventing absorption in the intestine.

Although activated carbons (ACs) have been recognized as one of the most effective and nontoxic adsorbents *(4)*, few studies about their employment as sorbents for AFB_1 are reported in the literature. Some authors observed low *(5, 6)* or no *(14)* ability of activated charcoal to reduce

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AFB_1 toxic effects in chickens. In contrast, detoxification tests carried out on ducklings *(28),* mink *(2),* and chickens *(13, 30)* showed that activated charcoal was effective in reducing the toxic effects of AFB_1. These contrasts in the data can be ascribed to the type of activated charcoal used.

No data on the efficacy of ACs in reducing AFB_1 carryover into milk are available. Galvano et al. *(12)* evaluated AFB_1 affinity of ACs in vitro, revealing very high adsorption and higher affinity for AFB_1 than HSCAS. The aims of our investigation were to evaluate the ability of ACs and an HSCAS to reduce the carryover of AFB_1 from feed to milk and to establish whether AC could be added to contaminated feeds in practical conditions.

MATERIALS AND METHODS

Sorbents

The following finely pulverized sorbents were used: two commercial ACs (CAC1 and CAC2) previously tested and demonstrated to have an high affinity in vitro for AFB_1 *(12),* and an HSCAS demonstrated to reduce aflatoxin toxicity *(10, 14, 20).*

Experimental design

According to a double-reversal experimental design *(34),* 12 multiparous late-lactation (30 to 33 weeks) Friesian cows were randomly distributed into three groups, each receiving only one type of sorbent and used as its own control. Cows were fed a basal diet (15 kg of corn silage, 4 kg of triticale (*Triticum secale*) silage, 4 kg of alfalfa hay, 2 kg of dehydrated beet pulp) to which 5 kg of pelleted feed containing AFB_1 naturally contaminated corn meal and copra was added. Compound feed samples of each dietary treatment were collected during the experimental period to determine the AFB_1 content.

The experimental period was divided into three weeks: during week 1, cows were fed diets containing AFB_1 alone; in week 2 the diets contained AFB_1 plus 2.0% sorbent, and in week 3 the diets again contained AFB_1 alone. AFM_1 excretion into the milk was determined only on days 3 to 7 of each week. Daily morning and evening milk samples from each cow were collected and mixed in proportion to morning and evening milk production. These samples were again combined in proportion to the daily milk production of each cow to constitute a representative bulk milk sample of each group. The AFM_1 concentration in the milk from cows fed sorbents were compared with those when sorbents were not fed. Moreover, a comparison between the three sorbents was carried out.

Methods of analysis

Determination of AFB_1 in feed was carried out according to the method of Howell and Taylor *(11)* as regards extraction and column cleanup; 250 µl of the purified extract were treated to convert AFB_1 to AFB_2A by trifluoracetic acid-catalyzed hydration according to Cohen and Lapointe *(3).*

AFM_1 in milk was extracted by immunoaffinity column (Easi-Extract™, Biocode Ltd, University Road, Heslington, York, UK) according to the method reported by Mortimer et al. *(17).* For both AFB_1 and AFM_1, the final extract was evaporated under a nitrogen stream and finally redissolved in the mobile phase (500 µl) and filtered (0.45-µm pore size); 50 µl of extract were usually injected.

Determination of AFB_1 in feed and AFM_1 in milk were carried out by high-performance liquid chromatography (HPLC) under the following experimental conditions. A Perkin-Elmer Series 4 HPLC (Perkin-Elmer Corp., Norwalk, CT, USA) equipped with ISS 100 sampling system and 1020 computing integrator was used. The detector was a Perkin-Elmer LS 4 fluorescence spectrophotometer with excitation and emission wavelengths of 365 nm (slit 15 nm) and 440 nm (slit 20 nm), respectively. The stationary phase was a LiChrosorb RP-18 (125 by 4 mm (i.d.) column) 5 µm (Merck, Darmstadt, Germany).

The mobile phase for AFB_1 determination was acetonitrile (A) plus water (B). The program was 20A plus 80B to 50A plus 50B, 0 to 12 min; 100A plus 0B, 13 to 14 min; and 20A plus 80B, 15 to 21 min. The flow rate was 1.5 ml/min. Retention time for AFB_2 A was 8.4–8.5 min. Mobile phase for AFM_1 determination was isocratic, acetonitrile-water (28:72). The flow rate was 1.5 ml/min. The retention time for AFM_1 was 5.4 to 5.5 min.

Statistics

AFB_1 and AFM_1 data are presented as means ± SEM and $P < 0.05$ values were considered to be significant according to the Duncan multiple range test *(7).*

RESULTS AND DISCUSSION

Owing to the addition of 2% AC the feeds become black, but no effects on feed intake were revealed, as the diets were entirely consumed. The addition of 2% CAC2 did not allow pelleting of feed because of the caking action of this carbon.

AFB_1 reduction in feed

The addition of ACs to the feed achieved a substantial reduction in the analytically detected AFB_1. As reported in Figure 1, the addition of CAC1 and CAC2 reduced detectable AFB_1 from 11.28 µg/kg to 2.68 µg/kg and 6.70 µg/kg respectively, whereas HSCAS reduced AFB_1 to 4.60 µg/kg.

Phillips et al. *(20)* in in vitro studies showed that HSCAS had a great affinity for AFB_1, whereas others *(32, 35)* reported a reduction of detectable AFB_1 in feed by the addition of bentonite. The authors explained these findings by the formation of a stable complex between the sorbents

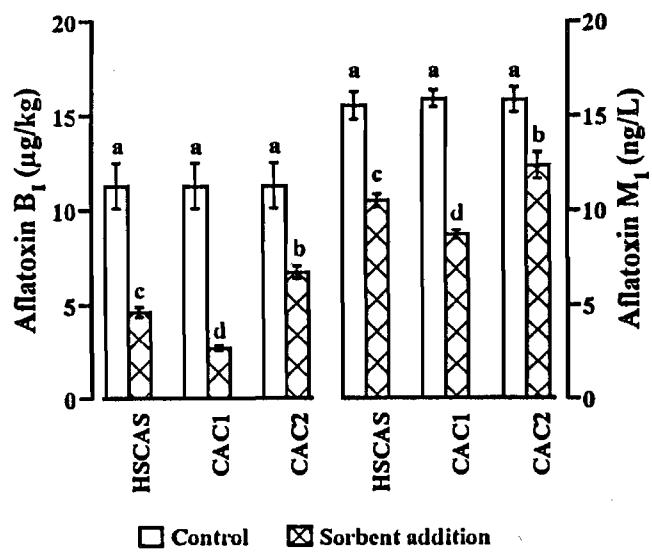

FIGURE 1. *Reduction of AFB_1 in feed and AFM_1 in milk as an effect of the addition of sorbents. Each bar represents the mean ($n = 4$ for AFB_1; $n = 10$ for AFM_1); error bars denote standard error of the mean. Different letters on the tops of bars mean significantly different values, $P < 0.05$.*

and AFB_1, resistant against solvent extraction. Since addition of ACs reduced the analytically recovered AFB_1 from the feed by 40.6% to 73.6%, the formation of an analogous inert, stable complex, AC-AFB_1, resistant to solvent extraction (chloroform), must occur.

Under the conditions of the present study, CAC1 showed greater reduction of the analytically recovered AFB_1 from feed than HSCAS (73.6% versus 59.2%) (Figure 1). This agrees with our previous study *(12)* in which in vitro affinity for AFB_1 of ACs compared to HSCAS was tested. In contrast, the performance of CAC2 was far from that expected. As pelleting of feed probably improves the adsorption of AFB_1 by ACs, the lower reduction of analytically recovered AFB_1 from feed with CAC2 could be related to the unsuccessful pelleting.

AFM_1 reduction in milk

The AFM_1 content of milk related to AFB_1 intake was lower than values reported by Munksgaard et al. *(18)*. Veldman et al. *(33)* reported that the average daily AFB_1 intake for dairy cows has to be below 40 μg/day to produce milk with less than 0.05 μg of AFM_1/kg (European Communities tolerance). In the present study, despite the average AFB_1 intake of cows being over 55 μg/day, the AFM_1 content of milk, with or without addition of sorbents was substantially below the EC tolerance level.

Mean values for AFM_1 of week 2 (sorbents present) were significantly lower than those of weeks 1 and 3 (Table 1). In agreement with the observations of Harvey et al. *(10)*, the absence of sorbents during week 3 allowed AFM_1 concentration in milk to reach the same or greater values than in week 1. Nevertheless, these results could be also related to the increase of AFB_1 intake in week 3. In contrast, AFM_1 secretion showed the same values from week 1 to week 3 because of decreased milk yield. However, data pooled for the 3 test weeks showed that both the ACs and the HSCAS were effective in reducing AFM_1 concentration in milk. In agreement with the results of analytic recovery of AFB_1 from feed, addition of CAC1 to feed caused a significantly higher decline of AFM_1 concentration in milk than HSCAS (45.3% vs 32.5%); furthermore, CAC2, with a 22.0% reduction, was less effective than CAC1 and HSCAS in reducing AFM_1 concentration in milk (Figure 1).

Carryover from feed to milk

Carryover of AFM_1 from feed to milk in weeks 1 and 3 (controls) ranged from 0.45% to 0.55%, whereas in week 2 (sorbents added) it ranged from 0.27% to 0.41%. Carryover data from the present study were similar to those (0.14% to 6.2%) commonly reported for dairy cows *(8–10, 15, 16, 18, 19, 24, 29, 31–33)*. Nevertheless, carryover values related to milk yield in both control and treatment weeks were quite low if compared to the values observed by other authors *(31–33)*. This could be partially explained by the late lactation stage of the cows, in agreement with the observations of Lafont et al. *(16)* and Veldman et al. *(33)*. Moreover, these latter authors *(33)* reported that a wide variability of carryover could be ascribed to animal factors related to the conversion of AFB_1 to AFM_1 by the mixed-function oxidase system.

The HSCAS was confirmed as a protective agent against AFB_1. Under our experimental conditions, 2% HSCAS addition reduced carryover by 36%. This reduction was lower than those reported in studies on dairy goats (2% HSCAS, 60% carryover reduction) *(10)*, (1, 2, and 4% HSCAS addition, 52, 82, and 87% carryover reduction, respectively) *(29)*, and on dairy cows (1% HSCAS, 44% carryover reduction) *(10)*, but higher if compared to the studies on diary cows of Harvey et al. *(10)* (0.5% HSCAS, 24% carry-over reduction), and Veldman *(32)*, who reported no carryover reduction by addition of 1% HSCAS. Nevertheless, differences in species, dosages of HSCAS and AFB_1, and duration of AFB_1 exposure should be considered when comparing published results.

We are aware of no reports on carryover reduction by addition of ACs. CAC1 was more efficient at reducing carryover than HSCAS (50% versus 36%). In contrast, CAC2 (27%) was less effective than HSCAS in reducing carryover. This latter result, as well as the lower reduction of detectable AFB_1 in feed and AFM_1 in milk, could be ascribed to the unsuccessful pelleting of feed. Our data

TABLE 1. *Reduction in carryover of AFB_1 in feed to AFM_1 in milk over a 3-week period in three groups of cows with or without sorbents added to the feed*

Week (group)	Sorbent in feed	AFB_1 intake (μg/day)	Milk yield (kg/day)	AFM_1 in milk (ng/l)[a]	AFM_1 in milk (μg/day)	Carryover AFB_1 to AFM_1 (%)	Reduction of carryover (%)
1 (1)	none	56.40	19.40	15.52 ± 0.73A	0.30	0.53	NA[b]
1 (2)	none	56.40	19.34	15.88 ± 0.44B	0.31	0.54	NA
1 (3)	none	56.40	19.66	15.83 ± 0.65B	0.31	0.55	NA
2 (1)	HSCAS	56.40	18.10	10.48 ± 0.33B	0.19	0.34	36
2 (2)	CAC1	56.40	17.72	8.68 ± 0.22C	0.15	0.27	50
2 (3)	CAC2	56.40	18.78	12.35 ± 0.70C	0.23	0.41	27
3 (1)	none	67.15	17.58	17.25 ± 0.73A	0.30	0.45	NA
3 (2)	none	67.15	17.66	17.83 ± 0.48A	0.31	0.47	NA
3 (3)	none	67.15	17.44	18.41 ± 0.69A	0.32	0.45	NA

[a] Mean ± SEM; $n = 10$. Within each group (see first column) of cows, values followed by different letters are statistically different ($P < 0.05$).

[b] NA, not applicable.

suggest that the complex AC-AFB_1 should decrease the bioavailability of AFB_1 for absorption in the intestine and could reduce carryover up to 50%. Measure of fecal AFB_1 excretion would provide additional evidence. Adding a lower amount (from 0.1% to 0.5%) may attenuate both pelleting difficulties and the color change of the feed. Since addition of ACs did not show effects on milk yield and composition or body weight (data not shown) our study demonstrates that ACs could be added to feed in practical conditions. As regards toxic effects related to addition of ACs, no obvious adverse effects were observed, but our data do not allow us to affirm that there would never be adverse effects on health status. Undesired preferential adsorption of useful organic molecules (e.g., vitamins or minerals) should be investigated in long-term studies.

In summary, our results demonstrate that ACs, high-affinity sorbents for AFB_1 in vitro, could reduce the AFM_1 content of milk to safe levels and are potential protective agents against aflatoxicosis at low contamination levels. Further in vivo investigations should establish lower amounts of ACs added to feed which can be efficacious. Moreover, studies in progress in our laboratory on the aflatoxin binding process could allow functional improvements to the adsorption ability of ACs.

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