Aflatoxin Inactivation: Treatment of Peanut Meal with Formaldehyde and Calcium Hydroxide

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ABSTRACT

A peanut meal contaminated with ca. 600 ppb aflatoxins was treated with formaldehyde alone and in combination with calcium hydroxide in a bench-scale reactor, operated both sealed and at atmospheric pressure. In general, thin layer chromatographic assays revealed that addition of calcium hydroxide to formaldehyde caused greater inactivation of the toxins than did formaldehyde alone. With the reactor sealed and 25% moisture in the meal, treatments for 1 hr with 0.5% and 1.0% formaldehyde plus 2.0% calcium hydroxide yielded products having 3 and 1 ppb aflatoxins, respectively, whereas under reflux at atmospheric pressure with 20% meal moisture, 1 hr treatment with 1.0% calcium hydroxide yielded a product with 5 ppb aflatoxins.

INTRODUCTION

The feeding of agricultural products that contain oilseed meals with high aflatoxin levels to farm and laboratory animals causes many detrimental physiological effects (1,2). Aflatoxin M_1, whose acute toxicity (3) and carcinogenic properties (4) have been recognized, was identified in the milk of mammals fed rations containing aflatoxins (5). When animals are fed rations containing sufficient amounts of aflatoxin to cause transmission into edible tissue, milk, or eggs, human health might be endangered.

Studies at the Southern Regional Research Center revealed that formaldehyde treatments appeared to inactivate aflatoxins in a contaminated peanut meal, and a limited investigation of this effect was reported (6). Also, a contaminated cottonseed meal was treated with formaldehyde alone and in combination with calcium hydroxide, and the products were evaluated chemically and biologically (G.E. Mann, A.N. Booth, L.P. Codifer, Jr., and S.P. Koltun, unpublished data).

Treatments of proteins or protein-containing dietary supplements with formaldehyde are reported to prevent microbial degradation of essential amino acids in the rumen, but permit their absorption in the abomasum or lower gut. Wool growth in sheep was stimulated when casein treated with formaldehyde was fed rather than untreated casein (7). Increases in the amount of crude protein disgested by sheep consuming peanut meal treated with formaldehyde, compared with untreated meal, have been noted (8). Experiments with ^{14}C-labeled formaldehyde fed to ruminants as an aldehyde-casein-oil complex revealed that ruminants effectively metabolize formaldehyde, which does not accumulate in either the carcass or the milk (9).

Treatment of oilseed meals containing aflatoxins with formaldehyde might achieve the desirable objectives of decreasing aflatoxin levels to minimize ill effects of animals consuming the meals, reducing the amount of aflatoxin M_1 in the milk of lactating ruminants, and allowing the animal to absorb more essential amino acids from the proteins in the meal.

Our objective was to determine the effects of a variety of conditions for treating a contaminated peanut meal with formaldehyde or with formaldehyde plus calcium hydroxide. On the basis of preliminary experiments, it was decided to limit the treatment periods to 1 hr and reagent concentrations to 2.5% or less, based on the *as is* meal wt. A bench-scale reactor was used for the treatments. The data gathered will be used for large-scale treatments to obtain sufficient material for biological evaluation.

EXPERIMENTAL PROCEDURES

All treatments were carried out in a 1-gal Model 150-R-J

[1] Deceased.

TABLE I

Treatments[a] of Contaminated Peanut Meal[b] with HCHO Alone and with $Ca(OH)_2$

Treatment number	HCHO (%)	$Ca(OH)_2$ (%)	Mean temp (C)	Aflatoxins in products (ppb)				
				B_1	B_2	G_1	G_2	Total
888	0.25	0.0	117	32	18	18	12	80
889	0.25	2.0	117	21	9	6	ND[c]	36
890	0.50	0.0	115	32	19	11	8	70
891	0.50	2.0	117	11	6	ND	ND	17
892	1.0	0.0	116	16	9	5	4	34
893	1.0	2.0	117	6	3	ND	ND	9
894	1.5	0.0	115	10	5	3	ND	18
895	1.5	2.0	117	5	2	ND	ND	7
896	2.0	0.0	115	3	3	Tr[d]	Tr	6+
897	2.0	2.0	116	3	2	Tr	Tr	5+
898	2.5	0.0	116	3	2	Tr	Tr	5+
899	2.5	2.0	116	2	1	ND	ND	4
900	0.0	0.0	116	33	14	8	13	68
901	0.0	2.0	117	19	7	ND	ND	26

[a] Reactor sealed, 15% moisture, 1 hr treatment periods.

[b] 570 ppb total aflatoxins.

[c] ND = none detected.

[d] Tr = trace (1 ppb or less).

TABLE II

Treatments[a] of Contaminated Peanut Meal[b] with HCHO Alone and with $Ca(OH)_2$

Treatment number	HCHO (%)	$Ca(OH)_2$ (%)	Mean temp (C)	Aflatoxins in products (ppb)				
				B_1	B_2	G_1	G_2	Total
876	0.25	0.0	116	27	19	11	12	69
877	0.25	2.0	116	8	0.5	ND[c]	ND	9
878	0.50	0.0	117	15	12	5	6	38
879	0.50	2.0	117	6	4	ND	ND	10
880	1.0	0.0	116	11	9	13	4	37
881	1.0	2.0	117	3	2	ND	ND	5
882	1.5	0.0	117	6	7	ND	ND	13
883	1.5	2.0	116	4	2	ND	ND	6
884	2.0	0.0	116	7	4	ND	ND	11
885	2.0	2.0	116	5	2	ND	ND	7
886	2.5	0.0	116	4	3	ND	ND	7
887	2.5	2.0	116	5	1	ND	ND	6

[a]Reactor sealed, 20% moisture, 1 hr treatment periods.

[b]570 ppb total aflatoxins.

[c]ND = none detected.

TABLE III

Treatments[a] of Contaminated Peanut Meal[b] with HCHO Alone and with $Ca(OH)_2$

Treatment number	HCHO (%)	$Ca(OH)_2$ (%)	Mean temp (C)	Aflatoxins in products (ppb)				
				B_1	B_2	G_1	G_2	Total
864	0.25	0.0	116	23	13	9	8	53
865	0.25	2.0	116	6	2	ND[c]	ND	8
866	0.50	0.0	115	19	14	3	5	41
867	0.50	2.0	116	2	1	ND	ND	3
868	1.00	0.0	114	4	4	ND	ND	8
869	1.00	2.0	116	1	Tr[d]	ND	ND	1
870	1.5	0.0	116	4	3	ND	ND	7
871	1.5	2.0	117	2	Tr	ND	ND	2+
872	2.0	0.0	116	3	2	ND	ND	7
873	2.0	2.0	116	2	2	ND	ND	4
874	2.5	0.0	117	3	2	ND	ND	5
875	2.5	2.0	116	2	2	ND	ND	4

[a]Reactor sealed, 25% moisture, 1 hr treatment periods.

[b]570 ppb total aflatoxins.

[c]ND = none detected.

[d]Tr = trace (1 ppb or less).

bench-scale reactor, steam jacketed, with a 1/4 hp, Type 4R totally enclosed drive (Bench Scale Equipment Co., Dayton, OH), and a Type A anchor impeller. The reactor cover had been fitted with two fixed baffle plates, and the vertical arms of the anchor impeller had been bent ca. 90° to move through the meal edge-on. Observations through the ports in the cover of the reactor indicated satisfactory agitation of 700 g of oilseed meal when the impeller rotated at 46-47 rpm. The reactor was operated in two different modes: 1) sealed with a Teflon gasket; and, 2) at atmospheric pressure under reflux. Zero time, recorded when the temperature in the reactor reached 100 C, usually required 5-10 min heat-up with 27-32 psig steam pressure in the jacket. When sealed, the reactor's internal pressure reached a maximum of 25-27 psig; internal pressure was atmospheric under reflux operation. All treatments were for 1 hr under the specified conditions.

An aflatoxin-contaminated peanut meal, designated CPM and containing ca. 230 ppb (μg/Kg) aflatoxin B_1 60 ppb B_2, 220 ppb G_1, and 60 ppb G_2, totaling 570 ppb toxins, was used for all treatments. CPM, 700 g, was weighed into a 3-qt bowl of a model C-10 Hobart Mixer. When calcium hydroxide (Powder No. 1372, J.T. Baker Chemical Co., Phillipsburg, NJ) was used, the specified percentage, based on *as is* meal wt, was added to the meal and the mixture agitated for 5 min. Then 37% formalin (Mallinckrodt No. 5014 USP formaldehyde solution [Mallinckrodt, St. Louis, MO]) and water were added in amounts calculated to give the specified percentage of anhydrous formaldehyde, based on *as is* meal wt, and to elevate moisture in the meal to the desired value. The mixture was agitated for 10 min and then transferred to the bench-scale reactor, where it was heated and stirred as described. After 1 hr treatment, the product was spread in a glass tray in a hood for air drying at ambient temperatures. A portion of the dried product was ground in an intermediate model laboratory Wiley Mill with a 20-mesh screen, and the ground material was assayed for aflatoxins according to Pons et al. (10,11). The usual precautions were observed in handling the aflatoxin-contaminated meal and apparatus (12).

When water, or water plus calcium hydroxide, was added to CPM to bring the moisture content to 20% or higher, the meal tended to form a thick, plastic mass, stalling the reactor's impeller. This effect was eliminated when formaldehyde was added together with the water, or with the water plus calcium hydroxide.

RESULTS AND DISCUSSION

Decreasing aflatoxin levels in 15% moisture-containing

TABLE IV

Treatments[a] of Contaminated Peanut Meal[b] with HCHO Alone and with $Ca(OH)_2$

Treatment number	HCHO (%)	$Ca(OH)_2$ (%)	Mean temp (C)	Aflatoxins in product (ppb)				
				B_1	B_2	G_1	G_2	Total
940	0.25	0.0	106	22	11	13	8	54
942	0.50	0.0	105	13	6	4	3	26
944	1.0	0.0	105	9	8	ND[c]	ND	17
946	1.5	0.0	105	5	3	3	2	13
948	2.0	0.0	105	4	4	ND	ND	8
949	2.5	0.0	104	3	2	ND	ND	5
955	0.25	1.0	105	17	6	8	4	35
950	1.50	1.0	104	12	5	4	3	24
951	1.0	1.0	105	8	4	3	2	17
952	1.5	1.0	105	5	3	2	2	12
953	2.0	1.0	105	4	2	2	2	10
954	2.5	1.0	104	3	1	3	ND	7
941	0.25	2.0	105	12	4	5	3	24
943	0.50	2.0	106	6	4	ND	ND	10
945	1.0	2.0	105	3	2	ND	ND	5
947	1.5	2.0	105	4	3	ND	ND	7
907	2.0	2.0	104	6	3	ND	ND	9
908	2.5	2.0	104	4	1	ND	ND	5

[a]Reactor under reflux, 20% moisture, 1 hr treatment periods.

[b]570 ppb total aflatoxins.

[c]ND = none detected.

CPM by treatments with various concentrations of formaldehyde, with and without 2.0% calcium hydroxide, are presented in Table I. The bench-scale reactor was sealed, hence mean temperatures of 115-117 C were obtained. Even without formaldehyde, the plastic mass was not formed when CPM of 15% moisture was used in the operation, making possible two control treatments, No. 900 (no reagents) and No. 901 (2.0% calcium hydroxide). As expected (13), heating with moisture alone caused considerable lowering of aflatoxins, which was enhanced when calcium hydroxide was present. The total aflatoxins remaining were above the FDA informal action level of 20 ppb (14). It is not known why the 0.25% formaldehyde treatments gave somewhat higher levels of aflatoxins than did the control treatments; it may have been due to a sampling error in selecting portions of meal to be treated. At each formaldehyde level, addition of 2.0% calcium hydroxide markedly lowered the aflatoxins, the effect being more pronounced at the lower formaldehyde levels. The use of 1.5% formaldehyde plus 2.0% calcium hydroxide under the treatment conditions of Table I appeared to be the lowest effective aldehyde concentration, yielding a product (Treatment No. 895) with a total aflatoxin content (7 ppb) well below the action level proposed by FDA.

Elevating the moisture content of CPM to 20% and using the sealed reactor yielded products with aflatoxin contents given in Table II. Somewhat lower aflatoxin contents were obtained, especially at the lower formaldehyde concentrations, and the enhancement effect of calcium hydroxide on aflatoxin inactivation was again greater at lower formaldehyde levels. Under these conditions, use of 1.0% formaldehyde plus 2.0% calcium hydroxide yielded a satisfactory product (Treatment No. 881, 5 ppb total aflatoxins).

As shown in Table III, aflatoxin inactivation increased markedly when the moisture was raised to 25% in the sealed reactor. In these treatments, 0.5% formaldehyde plus 2.0% calcium hydroxide (No. 867) yielded a product with only 3 ppb total aflatoxins.

Operating a sealed reactor under pressure would present a complication for large-scale treatments, hence experiments were performed under reflux at atmospheric pressure. Based on preliminary trials, 20% moisture content proved effective for these treatments, which are described in Table IV.

In the range of formaldehyde concentrations used, the addition of 1.0% calcium hydroxide did not, in general, result in any marked increase in aflatoxin inactivation. Use of 2.0% calcium hydroxide definitely improved inactivation to below the action level proposed by FDA (1.0% HCHO in No. 945, 5 ppb; 1.5% HCHO in No. 947, 7 ppb). If 2.0% calcium hydroxide is used, inactivation obtained with formaldehyde appears to be comparable with that obtained in the sealed reactor.

In all of these treatments, the aflatoxins G_1 and G_2 seemed to be more susceptible to apparent inactivation than the aflatoxins B_1 and B_2. Of the latter two, the B_1 generally appeared somewhat more stable towards treatments, especially at the lower formaldehyde concentration.

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