Inactivation of Aflatoxins in Peanut and Cottonseed Meals by Ammoniation

H.K. GARDNER, JR., S.P. KOLTUN, F.G. DOLLEAR and E.T. RAYNER,
Southern Regional Research Laboratory,[1] New Orleans, La. 70119

ABSTRACT

Aflatoxins in peanut and cottonseed meal can be inactivated by treatment with gaseous ammonia. In pilot plant runs, contaminated peanut meal was ammoniated at two levels each of moisture content, reaction time, temperature and ammonia pressure. Thin layer chromatography indicated that ammoniation inactivated the aflatoxins (121 ppb) in the meal to a nondetectable level. With a similar treatment, total aflatoxins (350 ppb) in cottonseed meal were reduced to 4 ppb. A series of runs was made with large scale equipment using cottonseed meal containing an average of 519 ppb total aflatoxins. Under optimum processing conditions, aflatoxin content of this meal was reduced to below 5 ppb and nondetectable levels.

INTRODUCTION

Although extensive work has been done to remove or inactivate aflatoxins in agricultural commodities, principally peanuts and cottonseed, a practical or economical procedure has not been developed up to this time.

It is known that laboratory and farm animals are adversely affected when fed rations containing sufficiently high levels of these toxins (1,2). In 1966, the Protein Advisory Group sponsored by the Food and Agriculture Organization, World Health Organization, and the United Nations Children's Fund recommended that the level of 30 μg/kg (30 ppb) of total aflatoxins not be exceeded in peanuts of protein supplements (3). More recently, the Food and Drug Administration of the U.S. Department of Health, Education and Welfare advised that a guideline of 20 ppb "would be used in routine regulatory actions beginning with the 1969 crop year" (4). To divert contaminated meals for use as fertilizer results in a loss of valuable protein supplements for animals. Moreover, the removal of these meals from the feed market represents a considerable economic loss to growers, processors and users.

It is therefore important to devise a practical procedure to inactivate or remove the aflatoxins present in contaminated oilseed meals. Numerous processes including solvent extraction and chemical treatments have been tried with varying degrees of success (5-18). However, most of these appear to be commercially impractical because of complexity of procedure, reduction in nutritional qualities, or economic unfeasibility.

Masri et al., (19) reported that ammoniation of aflatoxin contaminated peanut meal containing 709 ppb aflatoxin B_1, moistened to 9.6% and 14.6% at 200 F for 60 min under 20 psig anhydrous ammonia pressure, reduced the aflatoxin B_1 by 96.4% and 97.6%, respectively.

Feeding tests with these meals using two-day old ducklings eliminated bile duct hyperplasia and other liver abnormalities. After the first week, the mean body weight of the ducklings which were fed the ammoniated meals was about 30% higher than that of ducklings receiving rations of contaminated meal. This weight gain increased to about 50% after the second week. Dollear et al. (11) using a similar ammoniation procedure, but with lower temperature and shorter time, reduced the aflatoxin level of a contaminated peanut meal from 111 ppb total aflatoxins to less than 5 ppb. They also reported that the ammonia treated meal in the ration fed rats resulted in a protein efficiency ratio (PER) of 78% that of rats fed high quality peanut meal in their ration. Compared with the other procedures used, ammonia treatment produced a peanut meal of low aflatoxin content in the shortest treatment time and with only moderate alteration in chemical composition.

Cavanagh and Ensminger (20) have reported that ammoniated cottonseed meal is equal to regular cottonseed meal on an isonitrogenous basis for ruminants when evaluated in rate and efficiency of meat and milk produced. In this study, no case of toxicity was reported when cattle were fed up to six times normal levels of ammoniated cottonseed meal. The Food and Drug Administration has approved the use of ammoniated cottonseed meal in the feed of ruminants (21).

The present study was undertaken to determine the optimum pilot plant processing conditions to inactivate aflatoxins in oilseed meals by ammoniation and to translate these data to commercial scale equipment for evaluating processing efficiency and effectiveness. The large scale runs were also conducted to prepare a sufficient quantity of detoxified ammoniated cottonseed meal for biological testing, including two-year animal feeding studies.

EXPERIMENTAL PROCEDURES

Pilot Plant Scale Tests

Materials. The peanut and cottonseed meals used were produced by prepress solvent extraction. The peanut meal had a moisture content of 8.80%, as received, and a total aflatoxin content of 121 ppb. Other analytical data (moisture free basis) include: 1.30% lipids, 9.26% nitrogen, 5.2% crude fiber and 6.17% ash. The available lysine content (22) was 3.02 g/16 g N, and the nitrogen solubility in 0.02 N sodium hydroxide was 89.23%. The cottonseed meal had a moisture content of 9.86%, as received, and a total aflatoxin content of 334 ppb. Other analytical data

TABLE I

Pilot Plant Processing Conditions for Ammoniating Peanut Meal[a] and Residual Total Aflatoxin Contents, ppb

Time, min	Moisture content, %	Ammonia pressure, psig			
		15[b]	30[b]	15[c]	30[c]
15	9	61	30	41	5
	15	41	ND[d]	5	ND
30	9	44	14	24	ND
	15	26	10	5	ND

[a]Initial total aflatoxin content, 121 ppb.
[b]Temperature, 150 F.
[c]Temperature, 200 F.
[d]None detected.

[1]So. Utiliz. Res. Dev. Div., ARS, USDA.

TABLE II

Statistical Analysis of Data in Table I

Source of variance	Degrees of freedom, df	Mean square, MS	F-values
Moisture	1	1076	47[a]
Time	1	228	10[b]
Ammonia Pressure	1	2153	94[a]
Temperature	1	1336	58[a]
Interactions			
Moisture with time	1	163	7[b]
Moisture with ammonia pressure	1	173	8[b]
Time with ammonia pressure	1	86	4[c]
Ammonia pressure with temperature	1	144	6[b]
Moisture with ammonia pressure with temperature	1	133	6[b]
Error	6	22.9	6[b]

[a]Level, 99%.
[b]Level, 95%.
[c]Level, 90%.

(moisture free basis) include: 0.62% lipids, 7.06% nitrogen, 15.1% crude fiber, 6.3% ash, 0.07% free gossypol, 1.09% total gossypol, 5.90% total sugars and 0.11% reducing sugars. The available lysine content was 2.75 g/16 g N, and the nitrogen solubility was 60.79%.

The ammonia used was anhydrous ammonia having a purity (liquid phase) of 99.99% minimum and under a pressure of 114 psig (at 70 F) in the cylinder.

Equipment and Procedure. Ammoniating the meals was accomplished in a Groen portable stainless steel, steam jacketed pressure-vacuum reaction kettle of 10 gal capacity. The hinged cover was equipped with a removable agitator and variable speed drive. The agitator was designed to scrape the heating surface of the reactor and thoroughly mix the meals being treated. The cover and jacketed portion of the reactor were provided with a number of threaded openings, which could accommodate vacuum or pressure connections, purging systems, bleed-off fittings, etc. An indicating thermometer was installed in a port near the bottom of the reactor to measure the temperature of the meals being treated. Temperature was controlled by regulating steam and hot or cold water to the jacket as required. Gaseous ammonia under regulator controlled pressure was added to the meal through a fitting in the side of the reactor above the jacketed section. Nitrogen was used to purge the reactor of unreacted ammonia after each treatment.

To adjust the moisture content of the meal to be treated, water was added to the meal in a Model S-601 Hobart mixer equipped with stainless steel bowl and agitator and thoroughly blended.

Peanut meal, 15 lb, or cottonseed meal, 25 lb, was charged into the Hobart mixer, and a calculated quantity of water was gradually added to the meal while the mixer was operating. Blending continued for an additional 10 min to attain uniformity of moisture content in the meal. The meal, either hydrated or "as is," was transferred to the reactor which had been preheated. With constant agitation, heating of the meal continued until the temperature rose to 20 F below the desired reaction temperature. At this time, the steam to the reactor jacket was turned off, and gaseous ammonia was added to the reactor until the desired pressure was reached. With the addition of ammonia to the system, the temperature of the meal rose rapidly to the reaction temperature because of an exothermic reaction between the gas and the meal. The desired reaction temperature was maintained by alternately circulating cold water and steam through the reactor jacket. Reaction time was considered to begin when the selected reaction temperature and ammonia pressure were reached, and to end when the ammonia was vented. After venting the reactor, it was purged several times with nitrogen to remove any unreacted ammonia. The ammoniated meal was then transferred to trays, which were placed in a forced draft oven for 18 to 24 hr where the final traces of ammonia were removed, using ambient circulating air. The meal samples were then assayed for aflatoxin content by the method of Pons et al. (23,24).

Large Scale Tests

Materials. Two cottonseed meals were used in this series of experiments. One was a feed grade, prepress solvent extracted meal containing no detectable aflatoxin, obtained from Ranchers Cotton Oil in Fresno, California, for use as a control. The average meal moisture was 11.3%, as received. Other averaged analytical data (moisture free basis) include: nitrogen, 7.30%; crude fiber, 15.9%; lipids, 1.10%; free gossypol, 0.03%; and total gossypol, 1.04%. The available lysine content was 3.15 g/16 g nitrogen.

The other meal was a prepress solvent extracted meal and was specially selected because of its atypically high aflatoxin content, 450 ppb B_1 and 69 ppb B_2. No aflatoxins G could be detected. This meal was located at another source and shipped to Ranchers' plant for the ammoniation experiments to utilize the equipment which they had used previously to ammoniate cottonseed meal for ruminant feed. The average meal moisture was 9.2%, as received. Other average analytical data (moisture free basis) include: nitrogen, 7.02%; crude fiber, 13.2%; lipids, 0.93%; free gossypol, 0.02%; and total gossypol, 1.00%. The available lysine content was 2.91 g/16 g nitrogen.

Agriculture grade anhydrous ammonia gas was used for the ammoniation treatments. Food grade monocalcium phosphate anhydrous, $CaH_4(PO_4)_2$, "Stauffer V-90" was used to absorb the residual or unreacted ammonia remaining in the treated product after venting.

TABLE III

Pilot Plant Processing Conditions for Ammoniating Cottonseed Meal[a] and Residual Total Aflatoxin Contents, ppb

Time, min	Moisture content, %	Ammonia pressure, psig, ppb 15[b]	30[b]	45[b]
15	10	---	---	12
	15	81	69	4
30	10	---	---	7
	15	---	---	4

[a]Initial total aflatoxin content, 350 ppb.
[b]Temperature, 200 F.

TABLE IV

Effect of Processing Conditions on Aflatoxin Content of Ammoniated Cottonseed Meals[a]

Run No.	Meal charge, lb.	Time, min	Range, F	Average (Weighed), F	Ammonia pressure, psig		Total aflatoxin content of meal, ppb	
					Range	Average	Before ammoniation	After ammoniation
1	2500	30	243-261	251	45-50	48	519	3
2	2000	30	235-251	245	48	48	316	4
3	2000	30	206-254	238	48	48	496	1
4	2500	38	223-235	230	45-47	46	545	ND[b]
5	2500	30	205-237	224	47-48	48	ND	ND
6	2500	25	226-242	234	48-50	49	ND	ND

[a]Meal moisture adjusted to 12.5% before ammoniation.

[b]ND, none detected.

Equipment and Procedure

The reactor used at Ranchers Cotton Oil for ammoniation was a modified Schneckens dryer (18 x 3 ft diameter) capable of processing approximately 2500 lb. lots of meal and of operating at 50 psig pressure.

The reactor conveyor or agitator had been modified to a double ribbon type, with the outer ribbon having a right-hand flight and the inner ribbon having a left-hand flight. This configuration gave a forward and backward movement to the meal in the reactor. The conveyor was driven by a 30 hp motor and gear reducer and was operated at 40 rpm.

Water was added to the meal in calculated amounts using a rotameter. The reactor was heated with steam to the jacket. Temperature measurements were made with a dial thermometer located at one end of the reactor. The sensing element of the thermometer immersed approximately 3 ½ in. into the meal bed. Ammonia was supplied directly from a large storage tank. Keystone valves in the inlet and discharge ports and packing glands around the conveyor shaft retained the ammonia pressure in the reactor. However, it was necessary to repack the packing glands daily. A 3 hp centrifugal fan exhausted the vapors from the reactor after ammoniation. Auxiliary equipment included 9 in. diameter conveyors and rotalifts for loading and discharging meal.

A typical ammoniation run was conducted as follows: Cottonseed meal, 2000 or 2500 lb., was charged into the reactor. Samples for aflatoxin assay were taken from individual sacks of meal while charging. Using the rotameter, water was added in approximately 1 gal increments for 15 min to hydrate the meal to a level of 12.5%. When the addition of water was complete, blending and mixing were continued for 30 min. Steam (110 psig) was then introduced into the reactor jacket, and in approximately 35 min the meal temperature rose to about 160 F. Anhydrous ammonia was introduced into the reactor. A mild exothermic reaction occurred which accelerated the rate of heating to a temperature range of 235 to 250 F. Ammoniation was continued for 30 min after the ammonia pressure in the reactor reached the desired range of 45 to 50 psig. Steam to the jacket was then turned off and ammonia in the reactor was vented. The reactor was purged with air for 30 min using the centrifugal fan to exhaust excess ammonia. Approximately 75 lb of monocalcium phosphate was then added to the meal in the reactor, and the mixture was blended for an additional 15 min. The monocalcium phosphate absorbed residual quantities of ammonia in the meal resulting in an essentially odor-free product. The meal was then discharged from the reactor and bagged. Meal for aflatoxin assay was obtained by continuous sampling of the discharged product.

The ammoniated cottonseed meals and untreated cottonseed meal controls were assayed for aflatoxin content by the method previously cited (23,24), with the following exceptions: (a) Meal samples were extracted for 3 ½ min in a Waring Blendor rather than by extraction for 30 min on a wrist-action shaking apparatus. (b) A prewash of 150 ml of benzene-glacial acetic acid solvent (9:1 v/v) was used in the cleanup column operation (25) prior to the conventional ether-hexane wash and chloroform-acetone elution. This additional wash provided cleaner extracts for the final visual and densitometric determination of aflatoxin content. Densitometric measurements were made using the procedure of Pons et al. (23). In some instances, interfering substances precluded evaluation with the densitometer.

RESULTS AND DISCUSSION

Peanut Meal

Table I contains the factors or variables that were evaluated for the pilot plant scale ammoniation of peanut meal and the effect of these factors on the inactivation of the aflatoxin. The experiment was designed so that the effect of changing any one of the variables could be assessed independently of the others. For this experiment, the variables to be evaluated were moisture content of the meal to be treated, reaction time and temperature, and ammonia pressure. Each of these variables was also to be evaluated at two levels. Thus, 16 runs were conducted to determine the effect on the inactivation of aflatoxin by ammoniation at two levels of each of four variables. If the result of changing two or more factors or variables is to be studied, in general the most efficient method is to use a factorial design (26). An efficient method is one which obtains the required information with the required degree of precision and with the minimum expenditure of time and materials. The simplest class of factorial design is that involving factors at two levels, that is, the 2^n class, n being the number of factors. This is the class that has received most attention in the literature, and the theory has been extensively developed. For the purposes of this experiment, the factorial design was a 2^4 experiment.

The levels selected for each of the variables were based on some preliminary work, and recognizing the operational and economical limitations likely to be imposed on a commercial application of this procedure, the levels were considered to be realistic.

The data presented in Table I are rounded off to the nearest whole number. A statistical analysis of these data (unrounded values) using Yate's method (27) and an analysis of variance are shown in Table II. Ammonia pressure, reaction temperature, and moisture content of the meal were the dominant factors. Time was also important as were several two-way and three-way interactions. Among the significant interactions were all of the two-way terms involving ammonia pressure, moisture and temperature. The interaction of moisture and time was also significant. The

TABLE V

Chemical Analyses of Cottonseed Meals Before and After Ammoniation

Run No.	Nitrogen (MFB)[a] %		Nitrogen solubility, % (0.02 N NaOH)		Epsilon amino free lysine g/16 g N	
	B[b]	A[c]	B	A	B	A
1	7.06	8.18	68.2	48.9	2.67	2.41
2	7.06	8.18	68.2	50.0	2.78	2.41
3	7.06	8.18	68.2	49.2	3.29	2.41
4	6.90	8.43	68.2	50.6	2.89	2.45
5[d]	7.30	8.05	68.6	50.2	3.15	2.54
6[d]	7.30	8.05	69.5	48.9	3.15	2.54

[a]MFB, moisture free basis.
[b]B, before ammoniation.
[c]A, after ammoniation.
[d]Feed grade meal.

explanation and justification of the various significant interactions reported in Table II are beyond the scope of this paper and will be the subject of further investigation. Mere visual inspection of Table I shows that in all cases, when the ammonia pressure was increased, the residual aflatoxin content of the treated meal decreased. In seven of the eight cases, an increase in reaction temperature or in moisture content resulted in a decrease in the residual aflatoxin content; in the remaining case, there was no detectable quantity of aflatoxin at either level. Applying the nonparametric sign test, significance for all main effects is therefore indicated.

Cottonseed Meal

The processing conditions for the pilot plant scale ammoniation of prepress solvent extracted cottonseed meal and the aflatoxin assays of these treated meals are presented in Table III.

Based on the results obtained from the ammoniation of peanut meal, exploratory runs were made and it was decided to limit the reaction temperature to 200 F and increase the ammonia vapor pressure to 45 psig. As expected, the exploratory runs and those contained in Table III indicated that an increase in ammonia pressure or initial moisture content of the meal resulted in a greater decrease of aflatoxin. When cottonseed meal is treated at an ammonia pressure of 45 psig and temperature of 200 F, the aflatoxin content is lowered to well below the current FDA guideline of 20 ppb (4) in as little as 15 min even at a moisture content of 10%.

The large scale tests with contaminated cottonseed meal were conducted for two purposes. The first was to demonstrate that the pilot plant ammoniation procedures could be successfully applied to large quantities of meal in commercial scale equipment. The second was to prepare sufficient quantities of the ammoniated, aflatoxin inactivated cottonseed meal for long term feeding studies with the treated meal and suitable controls.

Following several preliminary runs with this equipment to establish suitable operating conditions, large scale runs were carried out in the modified Schneckens dryer. Four runs were made using aflatoxin contaminated meal, and two using feed grade, aflatoxin free meal. The processing conditions used in these runs and their effect on the aflatoxin in the treated meals are shown in Table IV.

It is evident from these data that inactivation of aflatoxin contaminated cottonseed meal by ammoniation can be readily achieved on a large scale with good reproducibility. In Runs 1-4, using comparable operating conditions total aflatoxin content was reduced from levels of several hundred parts per billion to less than 5 ppb.

As expected, the ammoniation treatment produced some changes in the chemical composition of the treated meal. Chemical analyses of the meals, before and after ammoniation, are shown in Table V. In general, some sacrifice in meal quality is evident. Nitrogen solubility, for example, which is frequently considered an index of nutritional quality for nonruminants, is lowered from 68% in the untreated meal to 50% in the ammoniated meal. Similarly, epsilon amino-free (EAF) lysine values drop from an average of 3.0 g/16 g N in the untreated meal to 2.5 g/16 g N in the ammoniated meal.

However, the total nitrogen content of the ammoniated meals averaged about 1% more than that of the untreated meal. This increase is viewed as an asset in the ammoniation treatment since this nitrogen "add on" may be utilized effectively in the diet of ruminant animals.

Animal feeding studies are currently in progress at the Western Utilization Research and Development Division, USDA to evaluate the physiological properties of ammoniated meals prepared in the large scale tests.

ACKNOWLEDGMENTS

Aflatoxin assays for pilot plant runs by L.P. Codifer, Jr.; technical assistance in the pilot plant by J. Lucas and W.J. Jackson; G.C. Cavanagh and J.D. McKinney of Ranchers Cotton Oil, Fresno, California, provided material, use of their equipment and gave technical assistance for the large scale runs; suggestions and advice by L.A. Goldblatt and H.L.E. Vix.

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[Received October 2, 1970]