Elimination of Aflatoxins From Peanut Meal

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Abstract

Peanut meal containing aflatoxins was heat treated in the presence of moisture and chemicals to reduce the aflatoxin content. Treatments with ammonia, methylamine, sodium hydroxide and ozone were effective in either destroying aflatoxins or greatly reducing aflatoxin levels as indicated both by TLC analysis and feeding experiments with ducklings and rats. Weight gains for animals receiving the treated meals were essentially comparable to those for animals receiving aflatoxin-free meal. The treated meals, however, had somewhat reduced protein efficiency ratios, as indicated by rat feeding tests. Complete elimination of aflatoxins from contaminated peanut meal was achieved by extraction with a 90% acetone–10% water (w/w) solvent system. The extracted aflatoxin-free peanut meal gave good growth in the duckling and rat feeding tests, and had a relatively high protein efficiency ratio.

Introduction

DURING THE PAST FEW YEARS some agricultural commodities have been found to contain toxic metabolic products which are produced by certain strains of the mold *Aspergillus flavus* (1) and by some other mold species. These metabolites, designated as aflatoxins, have been characterized chemically (2,3), and feeding studies have indicated that they have deleterious effects on some laboratory and farm animals if the toxins are fed at sufficiently high levels (4–7). The ingestion of aflatoxins by certain lactating animals has been reported to result in secretion of toxins in the milk (8,9).

Aflatoxins have been detected in peanuts and in the meal produced from this commodity (10). Aflatoxin-contaminated stocks of peanuts may be crushed to yield edible oil because conventional processing removes these toxins from the oil (11). The residual meal, however, retains the aflatoxins, and may be unsuitable for use in animal feeds. It has been proposed that this material be diverted to use as fertilizer (12). Such diversion lowers considerably the economic value of the meal and results in losses of valuable nutrients. Therefore, a research program has been initiated to investigate practical methods which might be employed to inactivate or remove the aflatoxins present in contaminated peanut meals. Previous work has indicated that heat treating peanut meal, either dry or in the presence of moisture, is ineffective in eliminating aflatoxin contaminants (13–16). Autoclaving wet toxic peanut meals at 15 psi (120 C) has been reported to result in a progressive reduction in toxicity with increasing time of autoclaving. However, the nutritional value of these treated meals is questionable (17). More recently, treatment of aqueous peanut meal slurries with hydrogen peroxide has been reported effective in achieving aflatoxin detoxification (18).

The effect of heat treating hydrated peanut meals in the presence of such reagents as ammonia, methylamine, sodium hydroxide (19) and ozone (20) to achieve a reduction or elimination of aflatoxins, and the effectiveness of solvent extraction of aflatoxins from contaminated meals using a 90% acetone–10% water (w/w) solvent system (19,21) have been demonstrated. The present investigation was undertaken to confirm the effectiveness of these treatments, and to prepare sufficient quantities for biological testing to establish freedom from objectionable levels of toxins and to measure nutritional quality of the proteins of the treated meals.

Materials

The peanut meal was a selected prepressed solvent extracted meal specially chosen because of its aflatoxin content. It contained 70 ppb (μg/kg) aflatoxin B_1, 30 ppb aflatoxin B_2, and 11 ppb aflatoxin G_1. No aflatoxin G_2 could be detected. The composition of this original meal is given in Table I.

The high quality peanut meal used as a control for the biological evaluations was prepared from shelled US grade 1 peanuts. These were rolled, cooked for 35 to 40 min to a maximum temperature of 104 C, then hydraulic-pressed.

Reagents used were: anhydrous ammonia 99.99% purity (The Matheson Co.), methylamine (Eastman 527, 40% in water), sodium hydroxide pellets (Baker Analyzed Reagent), and acetone 99% purity (Commercial Solvents Corporation). Ozone gas was generated by Welsbach Model T-23 laboratory ozonator supplied with a flow of air dried by passage through a silica gel column.

Treatments of meals with sodium hydroxide of methylamine were carried out in a 10 gal capacity Groen tilting-type reaction kettle constructed of 304 stainless steel. The reactor vessel was two-thirds steam jacketed along the sides and bottom to provide heating, and had a mixer fitted with Teflon scraper blades to provide efficient mixing of the meal charge. The agitator, powered by a ¾ hp motor, was operated at a speed of 72 rpm during all treatments. A reflux condenser fitted into the lid retained meal moistures at a constant level during treatments.

Ozone treatments were carried out in small batches in a bench-scale reactor that had been designed and constructed at this Laboratory (22). The reactor consisted of a jacketed 3 liter stainless steel vessel fitted with a gasketed cover and an agitator designed to efficiently mix the meal. The cover was fitted with a thermometer well and two ports, one to allow a reflux condenser to be inserted and the other to allow reaction gas to be introduced deep into the meal through a tube that extended to approximately 1.5 cm from the bottom of the vessel. Effluent gases from the reactor were vented to a hood via plastic tubing connected to the top of the reflux condenser. Constant temperature was maintained by controlling the flow of steam through the jacket.

Ammoniation treatments were carried out in a 25 gal capacity black iron pressure reaction vessel steam jacketed along the sides and bottom to provide heat. The reactor was equipped with a ribbon-type agitator operated at a speed of 30 rpm during treatment. The meal charge was placed into and removed

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TABLE I
Composition of Peanut Meals

	Controls		Inactivation-treatment				Extraction
	High quality meal	Original meal	Sodium hydroxide[b]	Methyl amine[b]	Ozone[c]	Ammonia[d]	90% Acetone–10% Water[e]
Moisture, %	6.41	7.22	6.86	8.86	6.30	7.90	9.74
Oil, % (MFB)	8.6	0.75	0.49	0.26	0.28	0.63	0.16
Nitrogen, % (MFB)	9.17	9.82	9.67	10.20	9.96	10.39	10.14
Crude fiber, % (MFB)	4.1	5.0	4.9	4.9	4.3	4.8	5.0
Ash, % (MFB)	4.65	5.34	6.86	5.41	5.23	5.43	5.67
Nitrogen, sol., %[a]	93.3	82.4	55.9	60.5	59.2	70.0	79.6
Avail. lysine, g/16g N	3.2	2.8	2.4	2.4	2.5	2.7	2.8
Total aflatoxins (ppb)	0.0	111.0	17.0	<5.0	18.0	<5.0	0.0

[a] In 0.02 N NaOH.
[b] For 90 min at 100 C.
[c] For 120 min at 100 C.
[d] For 15 min at 68 C, 42 psig.
[e] For 80 min at 50 C.

from the reactor by means of a perforated metal basket which retained the meal.

Acetone extractions were carried out using a batch-type pilot plant basket extractor. The single basket employed was 8 × 8 × 23 in. deep, with a tapered bottom, and a screen and gate valve. The extractor was fitted with a cover, and insulated to reduce heat loss during operation.

Methods

Hydration of the Meals

The following procedures were used to obtain the specific meal moistures described. Designated quantities of the peanut meals were blended in a Model S-601 Hobart mixer equipped with a stainless steel bowl and agitator, the calculated quantity of distilled water gradually added, and the blending continued for an additional 10 min. In the methylamine and the sodium hydroxide meal treatments, the required amount of reagent was dissolved in the water used to produce 30% meal moisture, thus assuring a homogeneous distribution of reagent throughout the meal. For the ammonia treatment, meal moisture was adjusted to the 15% level. Hydration of the smaller batches (700 g) of meal to 30% moisture prior to treatment with ozone was accomplished as described above using a Model C-10 Hobart mixer.

Methylamine Treatment

Peanut meal, 6.8 kg, was hydrated with 2.08 kg of distilled water to which had been added 213 g of 40% methylamine solution. The hydrated, reagent-laden meal was then transferred to the Groen reactor. Under constant agitation the temperature of the mixture was elevated to 100 C and maintained at this value for 90 min. The meal then was removed from the reactor, spread in shallow trays and allowed to air-dry at ambient temperature for at least 24 hr before it was assayed for aflatoxin. A final drying at 55 C for 1 hr reduced the meal moisture to 8.8%.

Sodium Hydroxide Treatment

Peanut meal, 6.8 kg, was hydrated with 2.22 kg of distilled water containing 136 g dissolved sodium hydroxide. The hydrated, reagent-laden meal was then transferred to the Groen reactor. Under constant agitation the temperature of the mixture was elevated to 100 C and maintained at this value for 90 min. The meal then was removed from the reactor, spread in shallow trays and allowed to air-dry at ambient temperature for at least 24 hr before it was assayed for aflatoxin. A final drying at 55 C in a mechanical convection oven for 1 hr reduced meal moisture to 6.8%.

Ammonia Treatment

Peanut meal, 6.8 kg, was hydrated with 625 g of distilled water to a moisture level of 15% and placed in the iron pressure reaction vessel. Under constant agitation, anhydrous ammonia was introduced into the vessel and maintained at a pressure of 42 psig and a temperature of 68 C for a period of 15 min. The ammonia pressure was then released by opening the vent valve, and the reaction chamber flushed thoroughly with nitrogen gas. The treated meal was then air-dried, without additional heat, in a mechanical convection oven overnight to a moisture level of 7.9%.

Ozone Treatment

Owing to the limited capacity of the laboratory ozonator to produce sufficient ozone to process one large quantity of meal, several smaller runs were made in the bench-scale reactor as follows: 700 g of the peanut meal was hydrated to a 30% moisture level and the hydrated meal transferred to the bench-scale reactor to be mixed and heated. When the meal temperature reached 100 C, approximately 25 mg of ozone per minute was introduced for a total gas effluent rate of 850 ml/min. This output was determined by wet test flowmeter reading and iodometric chemical titrations. The reaction was continued for a period of 120 min. The meal then was removed from the reactor, spread in a glass tray and allowed to air-dry at ambient temperature for at least 24 hr before it was assayed for aflatoxin. A final drying of the combined successive runs was conducted in a mechanical convection oven at 55 C for 1 hr to reduce the meal moisture to a level of 6.3%.

Acetone Extraction

Peanut meal, 6.8 kg, was simultaneously charged into the basket extractor with 10.2 kg of 90% acetone–10% water at 50 C. After 30 min, the solvent was drawn off and a second 10.2 kg of fresh solvent added to the meal charge. This second pass was retained for 10 min, then drawn off. Three additional 10 min extractions using 10.2 kg of solvent were carried out and the meal drained of residual solvent. Spreading the marc in shallow trays to dry at ambient temperatures overnight reduced the final moisture content to 9.2%. The total solids removed by this extraction procedure comprised 2.8% of the original charge of meal.

Aflatoxin Analysis

Meal samples were assayed for aflatoxin content by the method of Pons et al. (23). Briefly, the procedure involved extraction of the aflatoxins from the meals with 70% (v/v) aqueous acetone, purifica-

TABLE II

Mean Duckling Weights at One and Two Weeks on Treated and Untreated Peanut Meals

Meal	% In diet	Mean body weight (g)	
		1 week	2 weeks
High quality peanut	65	168	514
Aflatoxin contaminated	60	163	501
Sodium hydroxide treated	60	158	454
Methylamine treated	60	156	447
Ozone treated	60	139	410
Ammonia treated	60	155	453
90% Acetone extracted	60	174	536

tion by precipitation with lead acetate, partitioning of aflatoxins into chloroform, purification of the extract on a silica gel column, separations of aflatoxins on TLC plates coated with silica gel GHR, and visual evaluation of the intensity of fluorescence of test spots viewed under ultraviolet light.

Biological Assay

Biological assays for aflatoxin activity were conducted employing duckling feeding tests. The treated meals and aflatoxin contaminated peanut meals were each fed to 10 ducklings as 60% of the diet for two weeks. A high quality peanut meal was fed at a 65% level as a control. Weights were determined after one and two weeks and the livers removed for histopathological examination.

Protein Quality Evaluation

Protein efficiency ratio (PER) and protein retention efficiency (PRE) data were obtained using the rat bioassay method of Derse (24) with the exceptions that female rather than male rats were used, the assay was run for 10 rather than 28 days, and 5 rather than 10 rats per group were used.

Results and Discussion

Table I presents the composition of the treated peanut meals and control peanut meals used in this investigation. It is evident that the treatments described have substantially reduced, inactivated, or eliminated the aflatoxins from the contaminated peanut meal as measured by TLC assay. The aflatoxin content of all treated meals was well below a level of 30 ppb. Extraction with 90% acetone–10% water has proven most effective with a complete removal of the aflatoxins present.

The available lysine content of some of the treated meals has been reduced somewhat as indicated in the treatments with sodium hydroxide, methylamine and ozone. Nitrogen solubility values for these treatments are also lower than the original peanut meal. These reductions, however, cannot be attributed entirely to the reagents employed, since treatment times and temperatures in these instances are higher than those utilized for the ammonia treatment or the 90% acetone–10% water extraction.

Ammonia treatment produces a peanut meal of low aflatoxin content in the shortest treatment time, with only moderate alteration in chemical composition.

Based on the chemical data shown in Table I, extraction of aflatoxins with 90% acetone–10% water produces an aflatoxin free peanut meal which most nearly parallels the chemical composition of the original starting material.

The mean duckling weights in the feeding tests of treated peanut meals and untreated aflatoxin contaminated meal at 60% of the diet and the high quality peanut meal at 65% of the diet are presented in Table II. The best weight gains were observed with the 90% acetone–10% water extracted peanut meal whereas other treated meals gave lower weight gains than the high quality peanut meal and the aflatoxin contaminated peanut meal.

TABLE III

Summary of Rat PER and PRE Assays on Peanut Meals

Test diet	Protein efficiency ratio (PER), corrected	Protein retention efficiency (PRE)
Casein control	2.50	68.96
High quality peanut	1.91	52.48
Aflatoxin contaminated	1.82	52.48
Sodium hydroxide treated	1.36	47.36
Methylamine treated	1.31	45.28
Ozone treated	1.16	42.88
Ammonia treated	1.49	46.72
90% Acetone extracted	1.62	49.92

Microscopic examination of each of the livers from these ducklings showed that histopathological results associated with aflatoxicosis were present in 4 of 10 livers from ducklings fed the original aflatoxin contaminated peanut meal. The lesions were well defined but not extensive or severe. They could be considered 1+. None of the treated peanut meals produced any observable liver damage.

Protein efficiency ratios and protein retention efficiencies obtained in rat feeding tests are summarized in Table III. The PERs reported are average values corrected to compare with casein as a control at 2.50. Each meal was fed at a level of 10% protein to five weanling female rats. The PRE values are calculated net protein utilization (NPU) values, based on feeding tests but without analysis of the rat carcass for its nitrogen content.

A reduction in protein quality appears evident for some of the treated meals, particularly those treated at the higher temperatures with sodium hydroxide, methylamine and ozone. Of the treated meals, the 90% acetone-10% water extracted product exhibits the highest PER and PRE values. The poor growth of the ducklings and the low PER in rats resulting from the ozone treated meal suggests destruction of essential nutrients (amino acids) or production of toxic products, or both.

In general, the biological evaluations appear to confirm the chemical data obtained on these treated meals. It should be emphasized, however, that these biological tests are preliminary in nature, and more prolonged feeding experiments with these materials will be required for firm evaluations.

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