Use of urea for detoxification of aflatoxin

by

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A Thesis Submitted to the

Graduate Faculty in Partial Fulfillment of the

Requirement for the Degree of

MASTER OF SCIENCE

Department: Veterinary Pathology
Interdepartmental Major: Toxicology

Approved:

For the Graduate College

Iowa State University
Ames, Iowa

1991

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INTRODUCTION

Depending upon weather conditions, corn is susceptible to aflatoxin contamination. A large quantity of Iowa corn was contaminated during the 1988 growing year. Contamination of corn by aflatoxin is a major concern as it results in serious economic loss to farmers and threatens the public health. There are a variety of preventive and detoxification procedures available so that corn can be fed to livestock, poultry and swine and also could be incorporated into corn-based food products.

It has been estimated that in 1988 more than 20 % of Iowa corn exceeded the aflatoxin limit of 20 parts per billion of aflatoxin for interstate transportation, resulting in the restricted movement of such corn. Feeding of such corn to dairy cows resulted in the loss of ability to market milk for several dairies in Iowa. Questions are raised about the safety of feeding such corn to livestock, especially to poultry and swine. Therefore, detoxification of aflatoxin would help alleviate these problems and protect against the loss of a large market.

Several detoxification procedures are reported elsewhere including: roasting, pasteurization, steam heating, physical separation of affected seeds and chemical treatment. It is reported that the treatment of corn with gaseous ammonia reduced aflatoxin by 90-99%. This procedure is capital intensive

and is not always feasible under field conditions. Concerns of health risks such as chemical burns, ocular irritation and even blindness are raised when gaseous ammonia is used. There is also a possibility of corrosive effects of ammonia on metal bins and handling equipment.

In preliminary trials, treatment of high moisture milo with liquid urea resulted in release of ammonia and inhibition of mold growth in grain for 3-5 months. The effects of this method of ammonia generation on preformed aflatoxin in commodities has not been elucidated.

During silage preparation, urea is added to increase the protein content of corn silage for beef, sheep and dairy cattle. Ruminants have the ability to hydrolyze urea to produce ammonia which is incorporated into bacterial protein utilized by the animal.

Addition of appropriate levels of urea to silage could possibly reduce the aflatoxin level while the natural fermentation processes of ensiling produces sufficient organic acids to inhibit the growth of mold. Further, unhydrolyzed urea increases the available protein content of corn-based rations, which is an advantage of the system.

LITERATURE REVIEW

History of aflatoxin problems

The effects of aflatoxin contaminated foods and feeds on animals and humans has been reported widely (Park and Pohland, 1986; FDA, 1974; Keyl and Norred, 1978). Peanuts, corn, wheat, rice, cottonseed, copra, nuts, various foods, milk, eggs, and cheese have been contaminated by aflatoxin B_1, B_2, G_1, G_2, M_1 and M_2 (Anderson et al., 1975; Koltun et al., 1979; Kobbe et al., 1979). There are many animal species susceptible to aflatoxin including birds, fish and mammals. The toxin of major concern is aflatoxin B_1 (AFB_1).

Young animals of any species are more susceptible to the toxic effects of aflatoxin than older animals (Bilay, 1960). Some acute and chronic diseases can result by exposing humans or animals to aflatoxin contaminated foods or feeds. Because of their widespread occurrence and carcinogenic potential, aflatoxins have elicited the greatest public health concern (Busby and Wogan, 1984; Schuller et al., 1983; Van Egmond, 1987).

Aflatoxin causes severe economic losses (Park et al., 1984). The major clinical signs of intoxication appear to be anorexia, decreased weight gains, decreased egg production, hemorrhage and some increased susceptibility to infectious diseases. This is because aflatoxin has a very strong ability

to reduce the resistance of several animal species to bacterial, fungal, and parasitic infections (Keppler and deLongh, 1964; Butler, 1974).

Acute and chronic disease

Investigations have been made for acute aflatoxin effects in humans. When unseasonal rains and scarcity of food promoted the consumption of heavily molded corn with aflatoxin at 6000-16000 parts per billion (ppb), severe problems in human included death due to gastrointestinal hemorrhage (Butler, 1974).

Because aflatoxins, especially aflatoxin B_1 are very potent carcinogens in some animals, there is widespread interest in the effects of long-term exposure to low levels of this mycotoxin. Presently, only aflatoxin B_1 and sterigmatocystin are considered by the International Agency for Research on Cancer (IARC) as having produced sufficient evidence of carcinogenicity in experimental animals (van der Merwe et al., 1965).

Liver cell cancer (LCC) is one of the leading causes of cancer mortality in Asia and Africa. For example, in the Peoples' Republic of China, LCC accounts for approximately 100,000 deaths per year and is the third leading cause of cancer mortality. In parts of West Africa, annual LCC mortality can be up to 100 cases/100,000 population/year (Busby and Wogan, 1984).

It is believed that diseases caused by mycotoxins have been recorded some 5000 years ago. As a mycotoxin, aflatoxin is named from a combining of the words Aspergillus and flavus (Hesseltine, 1979). Twenty-three types of diseases have been strongly suspected to be aflatoxin related both in animals and man. It was not until 1960, that aflatoxin was discovered. After 1960, interest in mycotoxins developed at a tremendous rate, with literally hundreds of investigators in the field (Hesseltine, 1979). This resulted in the discovery of aflatoxin and recognition that aflatoxins and other compounds were potent carcinogens and teratogens (Miyake and Saito, 1965). Between 1960 and 1970, the study of aflatoxin underwent dramatic development. Aflatoxin has been identified structurally and its properties have been studied. Many researchers focused mainly on human food and animal feed. It has been shown that cows fed aflatoxin-containing feed excreted a toxic factor in their milk. A number of reports of liver disease in animals before 1960 were undoubtedly caused by aflatoxin (Purchase, 1977).

Aflatoxin is produced by Aspergillus flavus, one of three major toxin-producing genera of fungi: Aspergillus, Fusarium and Penicillium. As a toxic fungal metabolite, it occurs in a wide variety of substrates including feeds and foods. Aflatoxin impairs human health and has caused severe economic losses (Hsieh, 1979). There are four major aflatoxins: B_1, B_2, G_1, and

G_2 plus two additional metabolic products, M_1 and M_2, that are of significance as direct contaminants of food and feeds (Park et al., 1986).

Decontamination procedures

Prevention is the best method for controlling mycotoxin contamination. However, fungal contamination can occur before harvest as well as during harvesting and storage operations. Unfortunately, mycotoxins diffuse away from the mycelia and products having no visible evidence of mold damage can contain mycotoxins at significant levels; therefore, physical removal may not effectively detoxify the material (Goldblatt and Dollear, 1977; Jemmali, 1980; Jemmali, 1983).

There are only a few chemical approaches that can be applied for detoxifying or lowering aflatoxin levels from contaminated food and feed (Muller, 1984). The Food and Agriculture Organization (FAO) has outlined a set of criteria for determining the acceptability of a decontamination process which must be technically and economically feasible (Van Egmond, 1987).

Recently the most useful methods that are applied for decontamination of aflatoxin are: Ammoniation, Urea addition, High temperature, and Ozone.

Ammoniation appears to be the chemical method applied most extensively (Park and Lee., 1990). Ammoniation procedures designed to reduce aflatoxin levels in animal feeds can be carried out both at atmospheric pressure and ambient temperature and at high temperature and pressure (Dollear, 1969). The feedstuff is sealed in either plastic bags or sealed bins for 2-3 weeks. Temperatures as high as 100°C have been used, but most researchers set more natural experimental conditions which are at 21-43°C and storage for approximately 2 weeks at atmospheric pressure (Dollear et al., 1968).

Urea is used to generate ammonia under certain conditions. The addition of urease results in rapidly releasing ammonia which has been used to treat aflatoxin contaminated peanut flakes and soybean flour (Santi et al., 1982).

Methods of analysis

There are many methods for determining whether or not commodities are contaminated with aflatoxin. It is often necessary to determine the quantity of aflatoxin in a commodity to satisfy the Food and Drug Administration (FDA) requirement. Parts per billion (ppb), equivalent to nanograms/gram (ng/g), is the most common term for the description of significant aflatoxin concentrations.

Direct methods

Direct methods are those that analyze for aflatoxin chemically rather than relying on other products associated with aflatoxin contamination (Beckwith et al., 1976). Some of these methods are rapid, qualitative methods that determine the presence or absence of aflatoxin in the sample. Others are more rigorous, quantitative methods that provide for the accurate measurement of the amount of aflatoxin in the sample. The extraction of the aflatoxin from the sampled commodity is required for all these methods. Thin layer chromatography (TLC), minicolumn, high pressure liquid chromatography (HPLC) and immuno assays are the most current methods for the direct analysis (Dorner, 1990).

Indirect methods

Until recent years, chemical methods that were used to analyze samples for aflatoxin were generally time consuming, costly or unavailable. Therefore, indirect methods to determine the probable presence of aflatoxin contamination were developed. Visible A. flavus, bright greenish-yellow fluorescence (BGYF) or ultraviolet (black light) tests are the most common methods (Dorner, 1990).

Evaluation of ammonia decontamination

Nutritional properties

Of those decontamination procedures tested, ammoniation is the best practically applicable method (Fowler, 1979). Ammonia treatment can alter the composition of foods and feeds. Studies on nutritional quality of animal feeds as determined by chemical analyses revealed that there are some increased levels of total nitrogen, nonprotein nitrogen and soluble solids. Cystine, and sometimes methionine and nonreducing sugar levels as well as the nitrogen solubility index were reduced significantly. When ammonia treated corn was subsequently contaminated with mold, the mold converted the residual ammonia to protein or amide nitrogen. No significant changes were noted for starch and lipid components (Guthrie et al., 1981; Thiesen, 1977; Fowler, 1979).

Safety evaluation

Chemical assays on ammonia treated meals and aflatoxin analyses have determined that the toxin is reduced or changed because of ammoniation (Park and Lee., 1990). Those safety studies of the ammoniation procedures can be categorized into two major areas: Whole animal feeding studies of ammonia treated aflatoxin contaminated feeds, and toxicological properties of isolated aflatoxin related decontamination reaction products (Park et al., 1984).

Animal feeding studies

Acute and chronic toxicity feeding trials have been conducted with rats, mice, ducklings, swine, chickens, turkeys, lambs, and dairy and beef cattle (Goldblatt, 1971; Mann et al., 1971; Crosslin et al., 1979; Chelkowski et al., 1982).

Ducklings are the species with greatest demonstrated sensitivity to aflatoxin. Ammoniated aflatoxin contaminated feed produced no observable signs of aflatoxicosis or liver damage in the duckling test (Coker et al., 1983; Coker et al., 1985). Many studies have reported that aqueous ammonia effectively eliminated acute toxicity of aflatoxin in naturally contaminated whole corn and peanuts. Aflatoxin in contaminated meals could be reduced from 1000 to 20 ug AFB_1/kg or even lower by decontamination procedures (Ferrando et al., 1975).

The protein efficiency ratio (PER), quality, nitrogen solubility and lysine availability are important evaluation values. The PER values for ammonia treated meals were significantly lower, probably due to decreased nitrogen solubility and available lysine levels in the meals (Crosslin et al., 1979; Mann et al., 1971).

Animal weight and total serum protein results showed that there is no significant difference between control and ammoniated control diets after 2-4 weeks (Southern and Clawson, 1979; Southern and Clawson, 1980). Liver weights were elevated

for rats consuming diets containing aflatoxin over 1500 ug/kg. However, no significant differences were observed at aflatoxin concentrations less than 1500 ng/g (Norred, 1981).

Toxicity of decontamination reaction produces

A long term feeding trial with ammoniated corn has shown that visible nodules were found in livers of rats treated with aflatoxin contaminated diet (Kiermeier and Ruffer, 1974; Vesonder et al., 1975). Livers from rats fed ammoniated aflatoxin contaminated diet appeared normal (Lawlor et al., 1985; Lee et al., 1983). The safety of potential aflatoxin decontamination residues in human food was evaluated using relay toxicity feeding studies in rats. There was a nutritional effect but no evidence of carcinogenic or other toxic effects in these investigations (Lee et al., 1981).

Radio-labeled aflatoxin B_1 was used to study the relative toxicities of decontamination reaction products (Schroeder, et al., 1981). These isolates were tested by *in vitro* and *in vivo* test systems. In general, the ammoniation process is usually advantageous, resulting in increased levels of total and nonprotein nitrogen and protein, but reduced levels of sulfur containing amino acids, lipid, crude fiber and reducing sugar levels following ammoniation. The PER values were generally lower, probably due to decreased nitrogen solubility and available lysine levels.

The ammoniation process for aflatoxin decontamination has not been approved by Food and Drug Administration because of concerns about toxicity and potential carcinogenicity of reaction products in human food (Park and Lee, 1990). Specific decontamination procedures have been approved and put into use worldwide (Brekke et al., 1979). The States of Arizona, California, North carolina, Georgia and Alabama have permitted ammoniation procedures and provide information to farmers for ammoniation of aflatoxin contaminated corn and peanuts. Now ammoniation is routinely used in France, Senegal and Brazil and soon will be in Sudan. Several members of the European Economic Community import ammonia treated peanut meal on a regular basis (Park and Pohland., 1984).

MATERIALS AND METHODS

Trials and experimental conditions

Plastic cups[1] were used for incubations of ground feedstuffs and whole corn, except in trial I where plastic bags were used instead. Trials were done in sealed containers and containers were stored in sealed condition.

Moisture levels of ground and whole corn were analyzed. One hundred grams of feedstuff were accurately weighed and dried in a drying oven at a temperature of 100°C for 24 hrs. After this they were cooled in a desiccator and weighed again to a constant weight. The loss in weight was reported as % moisture in the feedstuff.

Trial I: Selection of optimum experimental conditions

A series of experiments was set up for selecting optimum experimental conditions from the variety of possible treatments. Ground corn was treated with urea at 0, 2, 4, and 8 grams per 200 grams of feed and stored in sealed plastic bags for 7 days and 14 days at room temperature (24°C). Moisture concentrations were 14% and 24%, thus resulting in 16 different treatments as follows (Table 1).

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[1]Polystyrene multipurpose container (Cat. # c8842-16), McGaw Park, IL 60085-6787.

Table 1. Selecting optimum experimental conditions from the variety of possible treatments

Moisture content	Incubation period	Urea added(g)			
	Days	0	2	4	8
14%	7	X*	X	X	X
14%	14	X	X	X	X
24%	7	X	X	X	X
24%	14	X	X	X	X

*Each treatment represents four replicates.

Trial II: Room temperature (24°C) incubation of ground corn and whole corn.

At 14% and 24% moisture levels, ground corn was treated with 0, 2, 4 and 8 grams of urea. Whole corn was treated with the same level of urea at 20% and 30% moisture and then was incubated for 14 days as shown (Table 2).

Trial III: Refrigerator temperature (0-4°C) incubation of ground corn and whole corn.

Ground corn at 14% and 24% moisture was treated with 0, 2, 4 and 8 grams of urea. Whole corn was treated with the same levels of urea at moisture contents of 20% and 30% and all samples were incubated for 14 days at 0-4°C as shown in Table 3.

Table 2. Room temperature (24°C) incubation of ground corn and whole corn

Feed	Moisture content	Urea added(g)			
		0	2	4	8
Ground corn	14%	X*	X	X	X
	24%	X	X	X	X
Whole corn	20%	X	X	X	X
	30%	X	X	X	X

*Each treatment represents four replicates.

Table 3. Refrigerator temperature (0-4°C) incubation of ground corn and whole corn

Feed	Moisture content	Urea added(g)			
		0	2	4	8
Ground corn	14%	X*	X	X	X
	24%	X	X	X	X
Whole corn	20%	X	X	X	X
	30%	X	X	X	X

*Each treatment represents four replicates.

Trial IV: Incubation of ground corn at 37°C.

Ground corn at 14% and 24% moisture was treated with 0, 4 and 8 grams of urea as indicated (Table 4).

Table 4. Incubation of ground corn at 37°C.

Moisture content	Urea added(g)		
	0	4	8
14%	X*	X	X
24%	X	X	X

*Each treatment represents four replicates.

Trial V: Treatment of aflatoxin contaminated corn (approximately 200 ppb) and highly aflatoxin contaminated corn (approximately 400 ppb) at 37°C incubation.

Untreated aflatoxin contaminated corn and highly aflatoxin contaminated corn were analyzed for their aflatoxin content and found to contain 220.8 ppb and 418.6 ppb, respectively.

Different levels of aflatoxin contaminated corn were treated at 24% moisture with 0, 1, 2 and 4 grams of urea respectively at 37°C. Experimental design was as follows (Table 5).

Table 5. Treatment of aflatoxin contaminated corn and highly aflatoxin contaminated corn at 37°C incubation

Aflatoxin level	Urea added(g)			
	0	1	2	4
Low	X*	X	X	X
High	X	X	X	X

*Each treatment represents four replicates.

Methods used to analyze aflatoxin and ammonia

Aflatoxin

The aflatoxin content of corn used in experimental trials was analyzed according to the General Mycotoxin Screening Method (Stahr, 1991). The procedure was divided into two parts.

1. Solvent extraction

Twenty five grams of ground corn was weighed from each experimental unit (cup). To this was added a mixture of 200 ml of acetonitrile:water (90:10, v/v) and the combination was immediately blended in a Waring blender for 4 minutes. The slurry was filtered through filter paper[2] and 100 ml of the filtrate was mixed with equal parts of petroleum ether in a 250 ml separator funnel for extraction. After this, 100 ml of

ferric gel (pH 4.6) was added and mixed well for extraction of color components that could interfere with thin layer chromatography (TLC). Approximately 100 ml of the lower layer of the separator funnel was collected and mixed with two volumes of 50 ml each of methylene chloride which was collected subsequently. The solution of methylene chloride was evaporated to dryness with nitrogen gas. The residues were dissolved with a small quantity of methylene chloride, transferred to a 2 ml vial and dried over a stream of nitrogen in preparation for analysis. Thus, the total amount of the aflatoxin in corn was subtracted 4 fold after the extraction process and taken into account for the final calculation.

2. Analysis of aflatoxin

To the dried aflatoxin extract vial, 200 ul of methylene chloride was added to dissolve the sample and this was mixed in a vortex mixer. Ten ul of this solution was spotted on a TLC plate[3]. The TLC plates were developed in chloroform:acetone (90:10, v/v). The developed plates were dried and scanned for fluorescence in a Fluorescent Densitometer[4]. Intensity of spots is directly correlated with aflatoxin content. The intensity of spots was measured as a function of peak height.

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[2]Whatman # 1 filterpaper, medium fast

Aflatoxin B_1 standard solution (10 ng/ul) was spotted on a TLC plate and developed under the same experimental conditions as that of samples.

An aflatoxin standard curve was obtained by plotting peak height (mm) scanned from the fluorescent densitometry of TLC plates versus ng of aflatoxin (Figure 1). Aflatoxin standard at 10 ng/ul was delivered to each of 4 spots in amounts of 2, 4, 6, and 8 ul giving aflatoxin amounts of 20, 40, 60 and 80 ug respectively. The data for this standard curve were obtained from the analysis of aflatoxin B_1 standard solution under the similar experimental conditions as that of the sample analysis procedures.

Table 6 shows the peak height of aflatoxin concentrations. The results were reported as a standard curve which was plotted as aflatoxin (ng) versus the peak height (mm). Response of peak height was proportional to the aflatoxin concentration. The resulting standard curve had little variation as shown by the small standard error values.

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[3]Whatman Fluorescent at 254 nm, 20 X 20 cms, 200 um layer octadecyl silane bonded KC 18F(catalog # 4803 800), purchased from P.J. Cobert Associates Inc., St. Louis, MO 63141.

[4]Kontes Fiber Optic Scanner, Spruce St. P.O.Box 729, Vineland, NJ 08360.

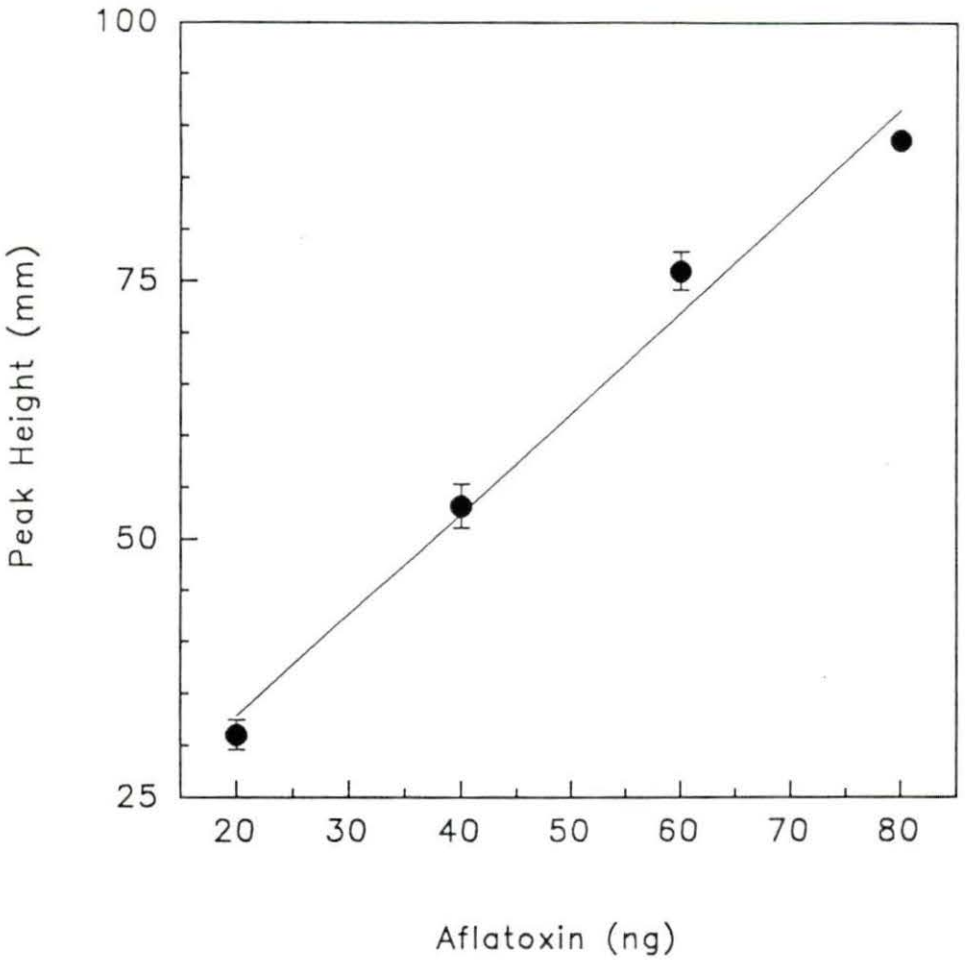

Figure 1. Aflatoxin standard curve. Standard curve of peak height vs. aflatoxin concentration was obtained by spotting a standard aflatoxin B_1 solution (10 ng/ul) in amounts of 2, 4, 6, and 8 ul

Table 6. Data from measurement of aflatoxin B_1 standard solution at the same experimental conditions as for sample analysis

Aflatoxin B_1 (ng)	Peak height (mm)				Mean	Std error
20	27.5	29.8	32.8	33.9	31.0	1.4531
40	51.8	54.2	58.5	48.3	53.2	2.1420
60	79.6	76.3	77.3	70.8	76.0	1.8659
80	88.3	86.8	90.1	88.8	88.5	0.6819

Analysis procedure for recovery of aflatoxin

Twenty five grams of feed was weighed and transferred into a Waring blender. A duplicate sample was weighed, transferred into another blender, and 25 ul of aflatoxin B_1 working standard solution (10 ng/ul) was added making a 10 ppb spike. The sample extraction and analysis procedures were the same as the procedures described above. Ground corn incubated at room temperature (24°C) was used to measure the spike recovery. Table 7 shows the results of a recovery study on different levels of urea treated aflatoxin contaminated corn and the average recovery. Spike recovery of 96.8% was obtained.

Table 7. Mean ± standard error of aflatoxin spike recovery for 24% moisture level of ground corn at room temperature (24°C)

	Urea (g)				Mean
	0	2	4	8	
Sample (ppb)	104.9*±2.8	96.7±5.3	53.4±1.7	11.5±3.3	
Spike (ppb)	114.9±2.6	106.6±2.2	63.1±7.3	20.7±4.7	
Recovery (%)	99.3	98.5	97.3	92.1	96.8

*Each treatment represents four replicates.

Ammonia analysis

Five grams of ground corn from each experimental unit (cup) was mixed with 100 ml of water and mechanically shaken for 30 minutes. The pH of the solution was adjusted to above 12 by using 10N sodium hydroxide (a few drops). The electrical potential (mV) value of the turbid solution was measured by using a digital pH meter[5]. An ammoniacal gas sensitive electrode[6] was used to analyze the amount of ammonia which was negatively correlated to the electrical potential (mV).

[5]Orion Research, 701A/digital pH , Cambridge, MA 02139.

[6]Fisher Scientific, Pittsburgh, PA 15238.

The electrical potential measured in all the experiments was a negative value. A blank containing distilled water has a positive electrical potential of 150 mV which was equivalent to 0.0045 ppm of ammonia. The ammonia content of the control sample was subtracted from the observed ammonia values of the treatments.

A series of ammonia standard solutions was prepared by diluting 1000 ppm stock ammonia standard solution[7] and analyzing for the electrical potential (mV) under the same experimental conditions as of the samples. Four replicates were measured at each ammonia concentration (Table 8).

The standard curve was obtained from the plot of millivolt reading versus log10 of ammonia concentration. The linear equation of the standard curve was based on the relationship of minus mV versus log10 ammonia concentration (Figure 2).

A calibration curve was constructed on semilog graph paper by plotting concentration (log scale) versus mV on the linear scale. A slope of -59 $\pm$ 4 mV with solutions measured between 20 and 30°C indicates correct electrode operation. This slope was obtained from experimental data and is very close to the instrument manual value of -58 mV for ideal theoretical electrode slope.

Table 8. Data from measurement of ammonia standard solution under the same experimental conditions as sample analysis

Ammonia (ppm)	Millivolts (mV)				Mean (mV)	Standard error
0.5	29.7	32.3	31.3	33.1	31.6	0.3663
1	13.1	13.5	13.0	13.2	13.2	0.1080
5	-26.8	-26.1	-24.7	-23.2	-25.2	0.7969
10	-42.8	-42.9	-44.7	-43.2	-43.4	0.4416
100	-103.3	-101.6	-101.7	-101.8	-102.1	0.4021

Urea analysis

Triplicate samples of corn treated with urea (8 grams at 0°C, 8 grams at 24°C, 4 grams at 37°C, and 2 grams at 37°C) were analyzed for urea by a commercial feed analysis laboratory[7]. Results were expressed as mean ± standard error for percent nonprotein nitrogen for each set of three samples. Results were obtained from the corn on a wet weight basis.

[7]Iowa Testing Laboratories, Eagle Grove, Iowa 50533

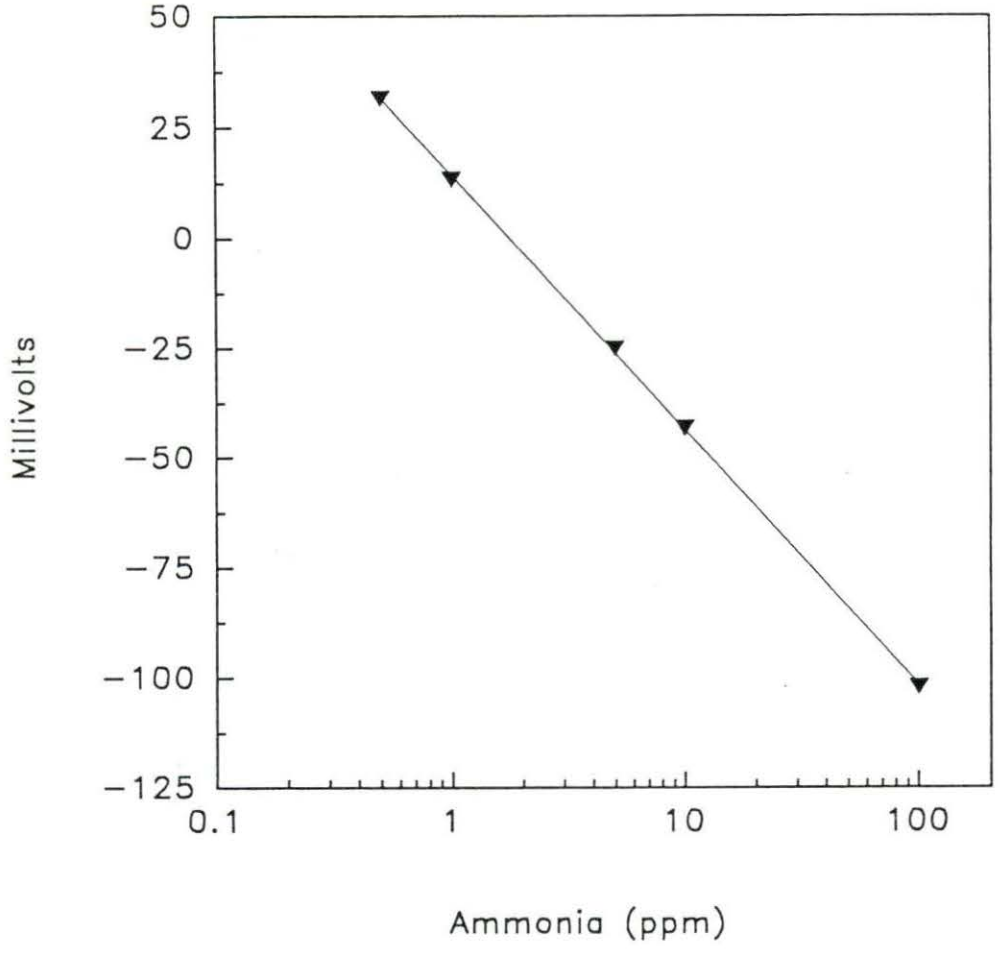

Figure 2. Standard curve for response of ammonia electrode in millivolts to ammonia concentration inparts per million (ppm). Values are means of four replicates of determinations with standard error not shown due to low variability in results

Materials used in experimental analyses

Chemicals

All chemicals and reagents used in this study were purchased from the same source (Table 9). They were all of reagent grade[8].

Chemicals were stored separately in original containers until appropriate solutions and standards were prepared. Ammonia standard solution was prepared fresh for each set of ammonia analyses.

Table 9. Chemicals used in experimental analyses.

For ammoniation	For aflatoxin analysis
Ammonia (1000 ± 5 ppm) standard solution prepared from ammonium chloride	Chloroform
Ammonia internal fill solution	Acetone
	Petroleum ether
	Ferric chloride
	Acetonitrile
	Aflatoxin B_1 standard solution (10 ng/ul)
	Methylene chloride

[8]Fisher Scientific, Pittsburgh, PA 15238

Instruments and equipment

TLC plates[3] were used to spot aflatoxin samples, standards and spikes.

An ammonia electrode[6] was used to measure the concentration of experimental solution. It was calibrated with a pH meter[5] to measure the mV which relates to the concentration of ammonia in ug/g.

A fluorescence densitometer[4] was used to measure the intensity of fluorescence in spots derived from the aflatoxin on TLC plates.

A Humidaire incubator[9] was used to incubate the samples kept at 37°C for 14 days.

Whole corn was ground in a Food processor[10] after the incubation period.

A concrete mixer[11] was used to mix the large quantity of untreated experimental corn. The mixer was adapted to rotate at 30 revolutions per minute.

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[9]Humidaire incubator, Model # 25, The Humidaire Incubator Co., Wayne St. New Madison, OH

[10]Dual-speed Food Processor, Hamilton Beach Inc., Washington, NC 27889.

[11]Montgomery ward, model # GIL 2471B Montgomery Ward Plaza, Chicago, IL 60610

Other materials

Number 2 yellow corn was used as control grain in all experiments. Aflatoxin contaminated corn was obtained from a farm in southeast Iowa which experienced a natural aflatoxin contamination. Contaminated corn was analyzed and separated into two groups (low and high), stored sealed in steel drums at 24°C, with levels measured as follows:

Low level aflatoxin	220.8 (ppb)
High level aflatoxin	418.6 (ppb)

RESULTS AND DISCUSSION

Trials

Trial I

In this experiment, treatment with 2 or 4 grams of urea for 7 days did not result in significant decrease in aflatoxin level, but treatment with 8 grams of added urea showed a significant decrease in aflatoxin level compared to controls (Table 10).

Table 10. Mean concentration ± standard error of aflatoxin (ppb) in Trial I after 7 or 14 days room temperature (24°C) incubation

H_2O (%)	Days	Urea(g)			
		0	2	4	8
14	7	$242.5^{[1]a}\pm10.9$	$224.3^{a}\pm12.8$	$211.2^{a}\pm13.0$	$153.6^{b}\pm6.6$
14	14	$248.9^{a}\pm3.8$	$240.0^{a}\pm10.6$	$60.8^{b}\pm11.0$	$17.6^{c}\pm2.7$
24	7	$236.8^{a}\pm6.7$	$214.4^{a}\pm2.1$	$204.8^{a}\pm0.9$	$122.2^{b}\pm6.6$
24	14	$166.0^{a}\pm5.3$	$163.2^{a}\pm5.6$	$112.0^{b}\pm9.7$	$6.4^{c}\pm1.8$

[a,b,c]Means that do not have a common superscript letter differ ($p< .05$).

[1]Represents mean of 4 replicates.

This indicates that the 7 days incubation is not enough time for ammonia to destroy aflatoxin at room temperature except when 8 grams of urea is used. Treatments of 14 days incubation showed that aflatoxin levels were decreased significantly as urea increased above 2 grams at both 14% H_2O and 24% H_2O.

Ammonia levels increased significantly with increasing urea at 24% moisture. Ammonia production at 14% moisture was not increased significantly with increased urea. The data show that there is no apparent difference between 7 days and 14 days incubation in ammonia generation at 14 % moisture (Table 11).

Table 11. Mean ± standard error of ammonia concentration (ppm) in Trial I after 7 and 14 days room temperature (24°C) incubation of ground corn

H_2O (%)	Days	Urea(g)			
		0	2	4	8
14	7	$55.9^{1a}\pm2.2$	$88.2^{b}\pm0.3$	$87.7^{b}\pm0.1$	$120.6^{c}\pm0.1$
14	14	$62.9^{a}\pm0.3$	$105.3^{b}\pm3.1$	$101.0^{b}\pm2.9$	$122.5^{b}\pm3.8$
24	7	$91.6^{a}\pm0.7$	$1262.2^{b}\pm0.4$	$2798.0^{c}\pm2.1$	$4751.0^{d}\pm0.9$
24	14	$976.4^{a}\pm0.3$	$1565.0^{b}\pm0.2$	$4547.0^{c}\pm2.7$	$9402.0^{d}\pm2.1$

[a,b,c,d]Means that do not have a common superscript letter differ ($p< .05$).

[1]Represents mean of 4 replicates.

At 24% moisture, there was a 10 fold increase in ammonia between 7 days and 14 days in the control group. This may be due to bacterial proteases resulting in generation of ammonia from protein in the corn. In every treatment at 24% moisture, ammonia generation was substantially higher at 14 days incubation than at 7 days incubation.

Aflatoxin level can be decreased more with longer incubation conditions than those of shorter time. Since the shorter time incubation was less effective at reducing aflatoxin level, the later trials focused on a 14 day incubation which is sufficient time for ammonia to lower the aflatoxin level.

Trail I also shows that treatment with 2 grams of urea is not sufficient to reduce the aflatoxin level.

Trial II

In trial II, after 14 days incubation of aflatoxin contaminated ground corn with 0, 2, 4 and 8 grams of urea at both 14% and 24% moisture content, aflatoxin and ammonia were analyzed. Treatments with 14% moisture content did not decrease the aflatoxin level significantly at any level of urea (Table 12). Treatments with 24 % moisture content decreased aflatoxin level significantly with increasing concentration of urea.

Table 12. Concentrations of aflatoxin (ppb) in Trial II after 14 days room temperature (24°C) incubation of ground corn

Moisture (%)	Urea(g)			
	0	2	4	8
14	291.2	278.4	275.2	265.6
	174.2	259.3	167.6	129.3
	172.5	206.9	213.5	152.9
	261.0	195.5	221.7	144.7
Mean ± SE[1]	$224.7^{a}\pm26.2$	$235.0^{a}\pm17.4$	$219.5^{a}\pm19.1$	$173.1^{a}\pm27.0$
24	79.1	80.8	56.9	33.2
	211.9	113.5	54.6	8.0
	38.2	79.1	37.5	1.8
	90.6	113.5	64.6	3.1
Mean ± SE	$105.0^{a}\pm32.4$	$96.7^{a}\pm8.4$	$53.4^{ab}\pm4.9$	$11.5^{b}\pm6.4$

[a,b]Means that do not have a common superscript letter differ ($p< .05$).

[1]Standard error of the means.

There was a large variation in the whole corn study at room temperature at 20 and 30% moisture. There is no significant difference among those treatments except treatment between 0 and 8 grams of urea at 30% moisture level (Table 13).

Table 13. Concentrations of aflatoxin (ppb) in Trial II after 14 days at room temperature (24°C) incubation of whole corn

Moisture (%)	Urea(g)			
	0	2	4	8
20	135.0	38.2	28.3	56.5
	261.0	0.0	166.0	22.1
	39.8	71.3	136.5	76.8
	0.0	3.7	15.2	23.8
Mean ± SE[1]	$109.0^{a}\pm50.3$	$28.3^{a}\pm14.5$	$86.5^{a}\pm32.9$	$45.9^{a}\pm11.5$
30	39.8	133.5	32.2	0.0
	178.1	131.6	44.7	0.0
	139.1	60.4	103.7	99.8
	101.4	116.8	127.0	39.2
Mean ± SE	$114.6^{a}\pm25.5$	$110.6^{a}\pm14.8$	$76.9^{ab}\pm19.8$	$34.7^{b}\pm20.3$

[a]Means that do not have a common superscript letter differ ($p < .05$).

[1]Standard error of the means.

Appropriate moisture in corn helps generate ammonia from urea. Urea treatment at 14 % moisture did not generate sufficient ammonia to lower the amount of aflatoxin in corn (Table 14).

Table 14. Mean ± standard error of ammonia concentration (ppm) in Trial II after 14 days room temperature (24°C) incubation

Moisture(%)	Urea(g)			
	0	2	4	8
Ground corn				
14	65.6[1a]±0.1	71.2[a]±1.8	89.8[b]±1.3	136.9[c]±2.6
24	32.9[a]±0.1	2204[b]±4.1	5083[c]±42.4	4149[d]±93.4
Whole corn				
20	164.5[a]±2.4	2689[b]±117.4	3203[c]±80.6	3018[c]±70.4
30	48.8[a]±0.7	7979[b]±46.2	8179[c]±52.0	8098[bc]±42.1

[a,b,c]Means that do not have a common superscript letter differ ($p < .05$).

[1]Represents mean of 4 replicates.

Trial III

Temperature could be another factor which may affect ammonia generation. Results of Trial III for aflatoxin confirm, as in Trial II, that treatment at lower moisture content (i.e. 14 % moisture) did not produce a decrease in aflatoxin content of corn at any level of added urea. Detailed results are in Table 15.

Table 15. Concentrations of aflatoxin (ppb) in Trial III after 14 days refrigerated temperature (0-4°C) incubation of ground corn

Moisture (%)	Urea(g)			
	0	2	4	8
14	195.5*	172.5	132.2	137.1
	273.5	182.4	222.3	164.3
	194.8	210.6	157.8	110.9
	186.2	230.8	110.3	163.4
Mean ± SE[1]	203.5±17.7	199.1±11.5	155.6±21.0	144.0±11.0
24	131.9	60.1	199.1	159.4
	131.3	76.8	119.1	137.8
	112.2	96.8	135.2	102.4
	130.2	245.6	135.2	64.4
Mean ± SE	126.4±4.1	119.8±36.9	147.1±15.3	116.0±18.0

*No significant differences among treatments at both levels ($P < 0.05$).

[1]Standard error of the means.

There was a large variation in aflatoxin content of whole corn stored at 0-4°C temperature with no tendency of reduction in aflatoxin concentration, even at treatment with 8 grams of urea. Thus reduction of whole corn aflatoxin

concentration at refrigerated temperature is toxicologically insignificant (Table 16). This means that use of urea to detoxify aflatoxin may be difficult during midwestern winters.

Table 16. Concentrations of aflatoxin (ppb) in Trial III after 14 days refrigerated temperature (0-4°C) incubation of whole corn

Moisture (%)	Urea(g)			
	0	2	4	8
20	28.4	54.2	74.2	135.2
	238.1	59.5	135.2	174.1
	168.6	0.0	91.6	61.1
	136.5	13.6	127.3	142.4
Mean ± SE[1]	$142.9^{a} \pm 37.8$	$31.8^{a} \pm 12.8$	$107.1^{ab} \pm 12.5$	$128.2^{ac} \pm 20.7$
30	20.2	185.6	203.0	293.8
	172.5	180.7	279.0	221.7
	480.6	120.8	329.2	126.7
	18.5	77.5	102.4	128.3
Mean ± SE	$172.9^{a} \pm 94.1$	$141.1^{a} \pm 22.4$	$228.4^{a} \pm 42.7$	$192.6^{a} \pm 35.0$

[a,b,c] Means that do not have a common superscript letter differ ($p < .05$).

[1] Standard error of the means.

Urea treatment at 24 % moisture for ground corn and at 30% moisture for whole corn for 14 days caused a significantly higher in the ammonia content than those at lower level of moisture corn relatively. The whole corn study showed that the ammonia produced by the treatments (2, 4 and 8 grams of urea) was increased at 30 % moisture. However 20% moisture did not give consistent dose related response for ammonia (Table 17).

Table 17. Mean ± standard error of ammonia concentration (ppm) in trial III after 14 days refrigerated temperature (0-4°C) incubation

Moisture (%)	Urea(g)			
	0	2	4	8
Ground corn				
14	$144.5^{1a}\pm3.9$	$202.5^{b}\pm3.6$	$202.1^{b}\pm0.1$	$248.8^{c}\pm1.4$
24	$114.1^{a}\pm0.9$	$323.2^{b}\pm5.6$	$448.0^{c}\pm0.8$	$562.6^{d}\pm1.2$
Whole corn				
20	$98.5^{a}\pm0.3$	$228.6^{b}\pm0.4$	$356.7^{c}\pm2.3$	$235.3^{b}\pm4.7$
30	$106.8^{a}\pm2.8$	$604.4^{b}\pm2.$	$542.8^{c}\pm0.0$	$683.1^{d}\pm8.5$

[a,b,c,d]Means that do not have a common superscript letter differ ($p< .05$).

[1]Represents mean of 4 replicates.

Trial IV

Results of trial IV indicate that urea treatment at 24% moisture significantly reduced aflatoxin level (Table 18). In some of the treatments, the level of aflatoxin was below detection limit.

Table 18. Concentrations of aflatoxin (ppb) in Trial IV after 14 days 37°C incubation of ground corn

Moisture (%)	Urea(g)		
	0	4	8
14	127.3	93.9	108.3
	191.2	95.2	129.3
	121.4	88.6	179.1
	70.9	67.7	187.5
Mean ± SE[1]	127.7^{ac}±24.7	86.3^{a}±6.4	151.0^{bc}±19.2
24	162.0	0.0	0.0
	129.3	5.4	0.0
	163.4	0.0	0.0
	125.0	0.0	0.0
Mean ± SE	144.9^{a}±10.3	1.3^{b}±1.3	0.0^{c}

^{a,b,c}Means that do not have a common superscript letter differ ($p < .05$).

[1]Standard error of the means.

However, treatment at 14 % moisture did not decrease aflatoxin content significantly. This suggests that higher moisture content is a necessary experimental condition for reducing aflatoxin level.

Trial V

Trial V showed that increasing levels of added urea to aflatoxin contaminated feed decreased aflatoxin content significantly (Table 19). This reduction is also expressed in percentage of decrease at different urea levels (Table 20).

Trial V demonstrated an increased production of ammonia at 37°C with increased urea, but the ammonia values were no higher than those produced at 24°C (Table 21).

Table 19. Mean ± standard error of aflatoxin concentration (ppb) in Trial V after 14 days 37°C incubation of ground corn (24 % moisture) with low and high aflatoxin level

	Urea(g)			
	0	1	2	4
ppb(low)	$132.7^{1a}\pm5.5$	$94.6^{a}\pm3.4$	$19.3^{b}\pm0.3$	$0.0^{c}\pm0.0$
ppb(high)	$217.3^{a}\pm0.5$	$180.7^{b}\pm4.0$	$162.2^{c}\pm6.0$	$85.7^{d}\pm7.4$

[a,b,c,d]Means that do not have a common superscript letter differ ($p < .05$).

[1]Represents mean of 4 replicates.

Table 20. Percentage reduction of aflatoxin after 14 days at 37°C incubation of ground corn (24% moisture) with low and high aflatoxin level

Level	Urea (grams)			
	0	1	2	4
Low	38.13	55.85	90.99	100.0
High	48.09	56.83	61.25	79.53

Table 21. Mean ± standard error of ammonia concentration (ppm) in Trial V after 14 days 37°C incubation of ground corn (24%) moisture with low and high aflatoxin level

	Urea(grams)			
	0	1	2	4
ppm (Low)	$70.4^{[1]a} \pm 0.3$	$1055^{b} \pm 31.0$	$2151^{c} \pm 43.0$	$3400^{d} \pm 5.5$
ppm (High)	$178.1^{a} \pm 2.6$	$1486^{b} \pm 9.0$	$2185^{c} \pm 3.9$	$4035^{d} \pm 2.1$

[a,b,c,d]Means that do not have a common superscript letter differ ($p < .05$).

[1]Represents mean of 4 replicates.

Ground corn study

Lower temperature incubation conditions (0-4°C) are not conducive to reducing aflatoxin content. Furthermore, lower temperature of incubation does not favor generation of ammonia from urea.

At room temperature (24°C) incubation, aflatoxin concentration can be reduced under certain conditions. Treatment of corn with 4, and 8 grams of urea reduced aflatoxin content significantly when the corn contained 24 % moisture but not at 14% moisture (Figure 3). This could be because of increased production of ammonia at 24% moisture since moisture is needed for hydrolysis of urea to ammonia. Incubation period is also an important factor for aflatoxin destruction. Incubation for 7 days did not effectively reduce aflatoxin content. Treatment with 8 grams of urea for 7 days was associated with a slight but insignificant reduction in aflatoxin. Such concentration of urea is very high and therefore its applicability is limited.

Possible reasons for room temperature incubation not reducing aflatoxin content are: (1) The room temperature does not favor maximum release of ammonia from urea. Therefore, higher temperature incubation could be better for ammonia production, especially for high moisture corn.

(2) Room temperature could be the best condition for aflatoxin production. Aflatoxin production may be optimal at temperatures that remain above 24°C (Tuite, 1979). At both levels of moisture (i.e. 14% and 24%), aflatoxin content could potentially be increased during the incubation period. However, at 14% moisture growth and toxin production of Aspergillus flavus is not likely. While ammoniation can reduce the aflatoxin level, there could be simultaneous production of aflatoxin at high moisture levels which may balance a slight reduction of aflatoxin by ammoniation with lower amounts of urea.

Incubation at higher temperature (37°C) is the best condition for aflatoxin reduction as higher temperature incubation is better for ammonia generation from urea. Also the 37°C temperature incubation appears to retard the formation of aflatoxin (Figure 4).

Trial V showed that treatment of corn with 2 and 4 grams of urea reduces aflatoxin significantly at moisture contents of 14% and 24 %. This indicates that ammonia was fully generated during the higher temperature incubation. Also the chemical reactions involved in ammonia destruction may proceed more rapidly and completely at higher temperatures.

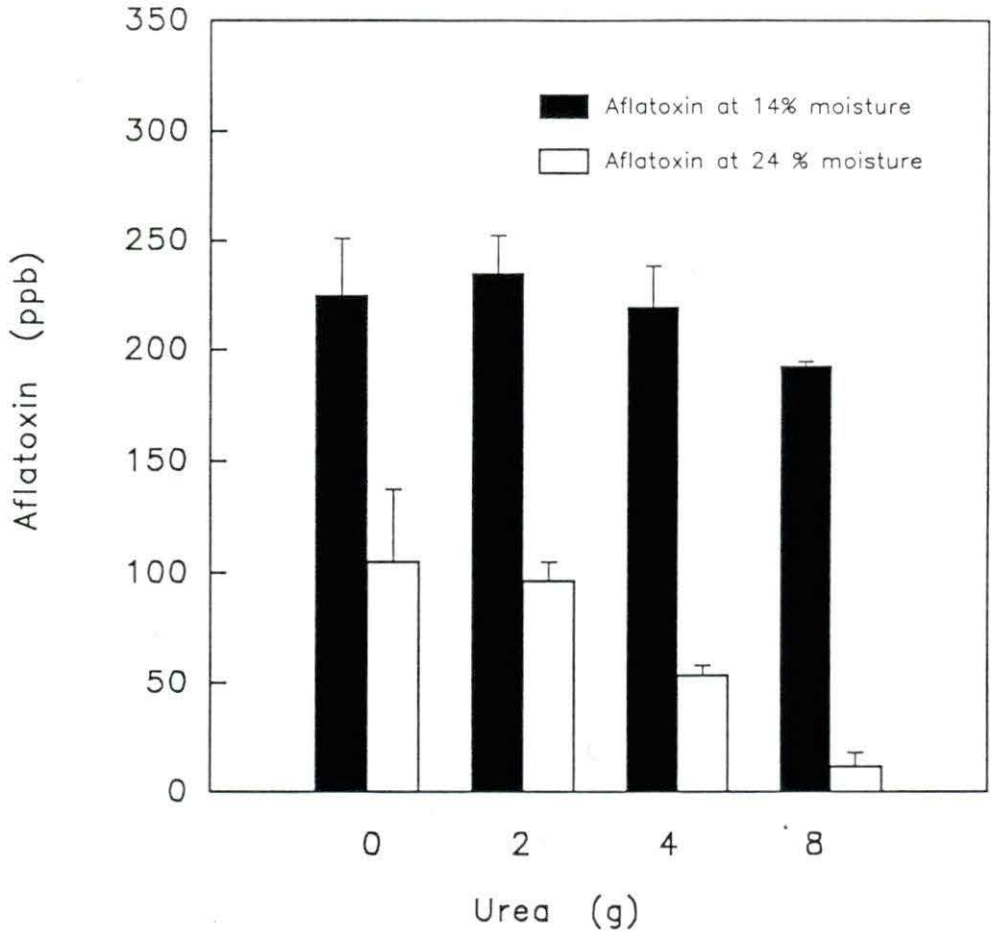

Figure 3. Aflatoxin concentrations (means ± SEM) after 14 days incubation with different amounts of urea. Urea was added to 200 grams of ground corn and incubated for 14 days at 24° C

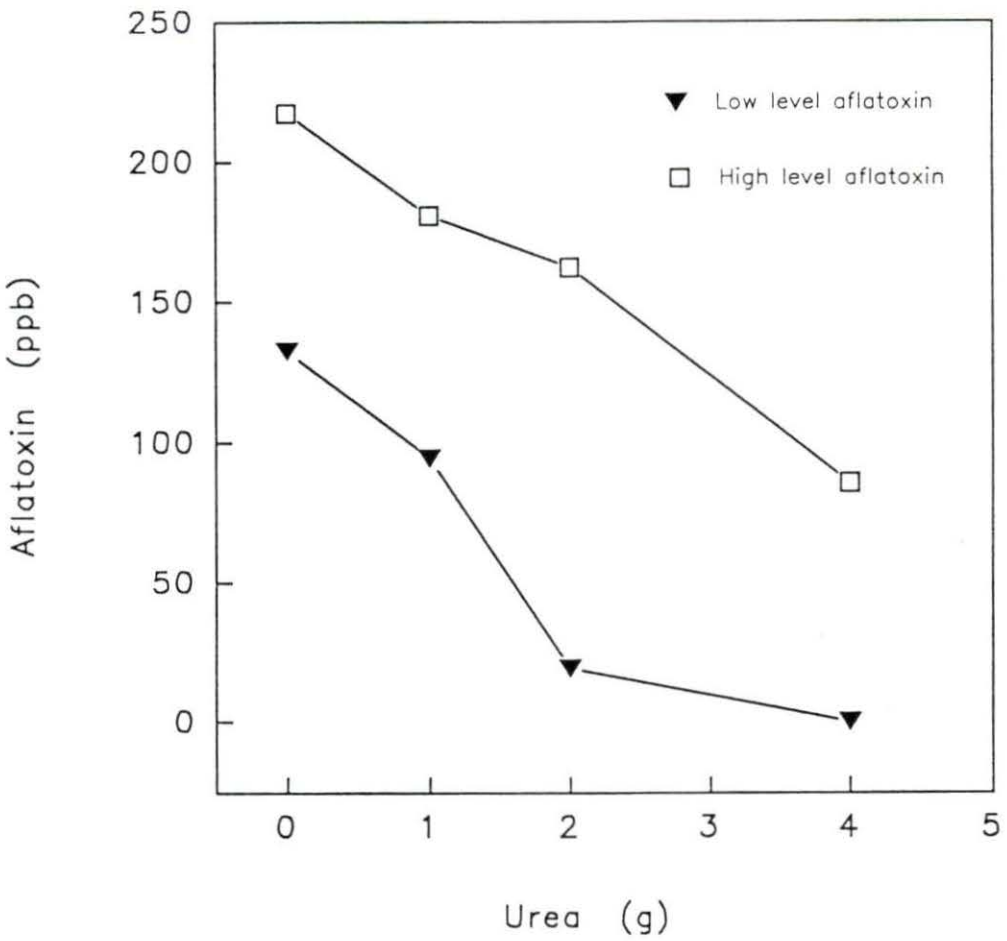

Figure 4. Concentrations of aflatoxin in response to increasing amounts of urea. Aflatoxin concentrations were 220.8 ppb (low) and 418.6 ppb (high). Urea was added to 200 grams ground corn

Treatment with 4 and 8 grams of urea in Trial IV showed that the aflatoxin level is extremely low and undetectable after 14 days incubation. Under this situation aflatoxin was almost completely destroyed (Figure 5).

Acidity (pH) values were measured immediately after the water extraction procedure for ammonia determination (Table 22). Because pH values relate to ammonia concentration in each treatment, the measurement of pH value has a qualitative significance for predicting ammonia production. Results revealed that pH > 8 always was associated with ammonia values over 2000 ug/g, whereas pH < 6.2 was associated with ammonia values less than 100 ug/g (Figure 6).

Table 22. pH versus ammonia concentration in extracts from corn treated with urea

pH	Ammonia (ppm)
6.07	65.6
6.13	71.2
6.18	89.8
6.24	136.9
8.26	2204.0
8.58	4149.0
8.69	5083.0

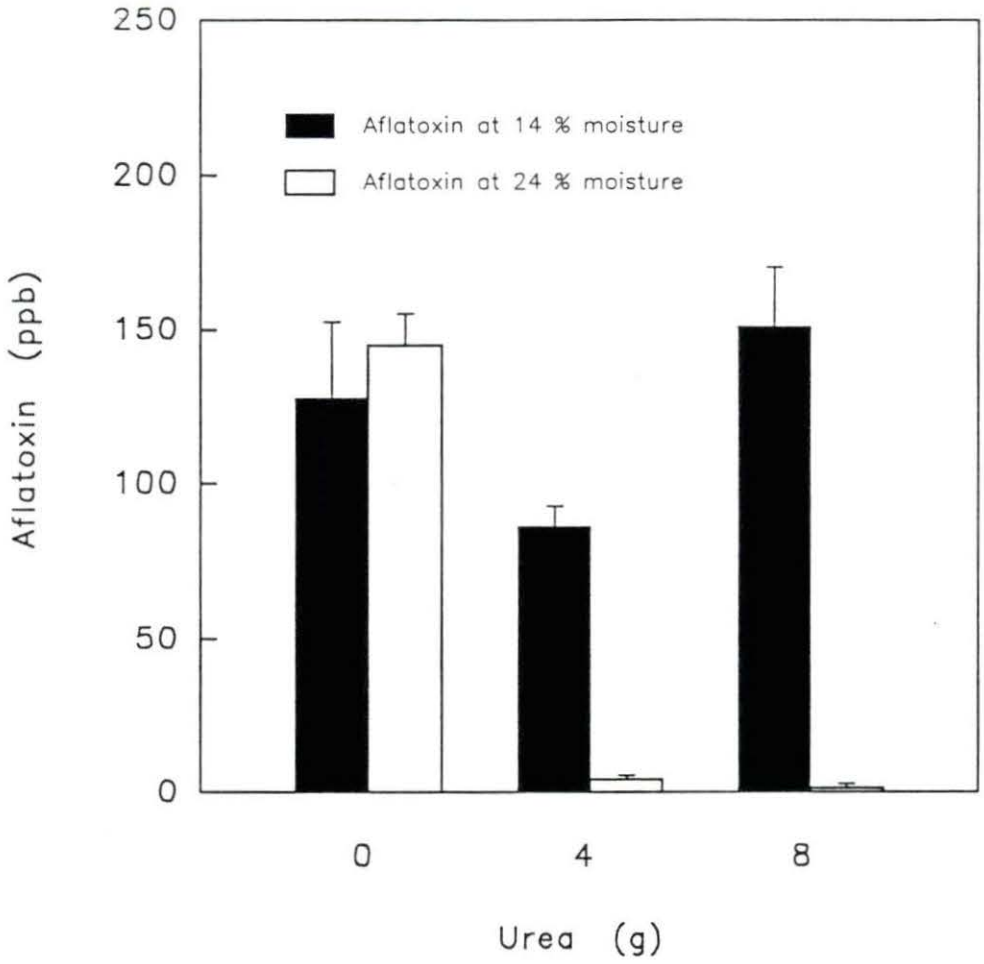

Figure 5. Aflatoxin concentrations (mean ± SEM) after incubation with urea at 37° C. Urea was added to 200 grams ground corn at moisture concentrations of 14% and 24%

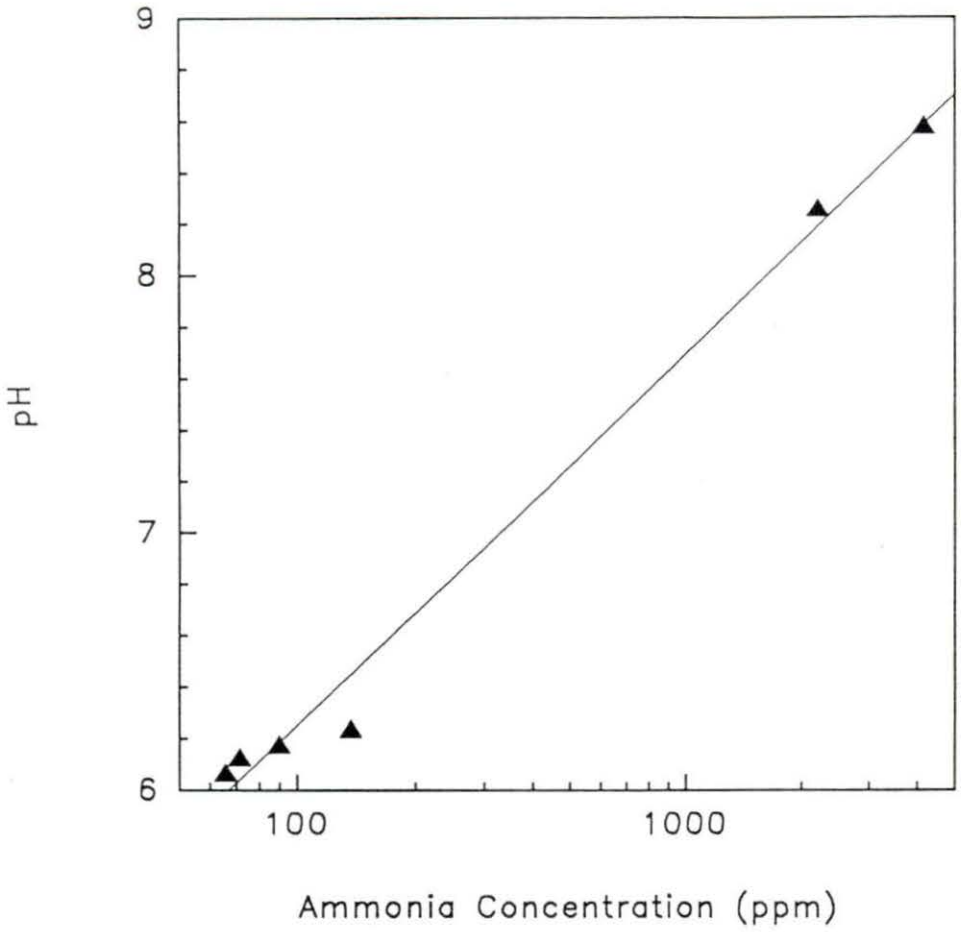

Figure 6. Response of pH to ammonia concentration in corn treated with urea. Results were obtained at 25° C after incubation of urea and corn for 14 days

As a fast and simple method, the determination of pH value would be a good practical method for farmers to check effectiveness of ammoniation although its value would not completely predict aflatoxin concentration in each situation of urea treatment of contaminated corn.

Whole corn study

Results of the whole corn study revealed a wide variation in the data after analysis. The possible explanation for such fluctuation is non-uniform distribution of aflatoxin in whole corn. Even after proper and thorough mixing of whole corn, there still exists a wide variation which may be derived from highly contaminated single kernels (Lillehoj, 1979). Consequently, comparing treatments in whole kernel corn would best be done in a large scale experiment where high level contamination of individual kernels will have less effect. This would be a subject for further research.

Mold study

After 14 days of refrigerated temperature (0-4°C) incubation at both 14 and 24 % moisture content, there was no mold growth. At room temperature incubation and 14% or 24 % moisture content for 7 days, corn was not moldy. Also, at 14 % moisture content for 14 days it was not moldy. Treatment with 8 grams of urea and 24 % moisture for 14 days incubation prevented mold growth.

However, 0 and 2 grams of urea at 24 % moisture and 14 days incubation allowed abundant mold growth while treatment with 4 grams of urea resulted in a moderately moldy condition.

Samples from Trial V were submitted to a commercial laboratory[12] for mold counts and mold identification (Table 23).

Table 23. Total mold count (CFU/g) and Aspergillus flavus in urea treated ammoniated corn at both low and high level of aflatoxin contaminated corn

		Urea (g)			
		0	1	2	4
Mold count (CFU/g)					
Low	A[1]	$3X10^6$	$4X10^6$	<10	<10
	B	$3X10^6$	$1X10^4$	<10	<10
High	A	$3X10^6$	<10	<10	<10
	B	<10	20	<10	<10
Aspergillus flavus (CFU/g)					
Low	A	$2X10^6$	$4X10^6$	ND[2]	ND
	B	$2X10^6$	$9.7X10^3$	ND	ND
High	A	$2X10^6$	ND	ND	ND
	B	ND	20	ND	ND

[1]Samples A and B are duplicate treatments.

[2]No A.flavus detected.

[12]Woodson Tenent Laboratories, Des Moines, Iowa 50313

Higher temperature (37°C) treatment with 0 and 2 grams of urea resulted in moderate mold contamination, but treatment with 4 and 8 grams of urea allowed no mold growth at all moisture levels for 14 days incubation.

Higher moisture content and higher temperature(37° C) are two parameters which could allow mold growth but not produce aflatoxin. The longer time of incubation with urea at room temperature resulted in less aflatoxin remaining. This perhaps could be due to more release of ammonia with longer time incubation. The mold growth can be controlled by higher levels of ammonia. Higher moisture content and higher temperature incubation of corn is more likely to cause mold growth, but treatment with higher concentrations of urea (4 grams or more per 200 grams of corn) results in the prevention of mold growth and reduction of aflatoxin level.

Urea analysis

Urea concentrations in the four conditions tested (8 grams at 0°C, 8 grams at 24°C, 4 grams at 37°C, and 2 grams at 37°C) were 9.6 $\pm$ 0.25, 10.83 $\pm$ 0.79, 6.05 $\pm$ 0.36, and 3.37 $\pm$ 0.20 respectively. The treatments which resulted in greater ammonia releases (higher incubation temperatures) were not proportionally lower than those where less ammonia was released. However, urea remaining was proportional to urea added.

CONCLUSIONS

A longer period of incubation (14 days) at higher temperature (37°C) is most likely to decrease aflatoxin significantly when urea is used for ammoniation procedures.

Moisture content appears to be one of the crucial experimental factors for ammoniation and detoxification. Higher moisture level (24%) promotes detoxification of aflatoxin contaminated corn even at room temperature (24°C) conditions.

It is possible to prevent mold from growing during the incubation period by increasing the urea level added to aflatoxin contaminated feedstuff, especially for higher levels of moisture content because mold growth appeared to be ammonia dependent.

Lower temperature incubation (0-4°C) for ammoniation appeared to be an experimental condition which would not change aflatoxin level significantly, possibly because the chemical reaction between ammonia and aflatoxin could be slowed dramatically at lower temperatures.

Further studies should focus on finding more precise experimental conditions to reduce aflatoxin level, minimize the use of urea, and prevent mold from growing. Further understanding of those factors causing aflatoxin generation may help experimental design of detoxification procedures.

LITERATURE CITED

Anderson, H. W., E. W. Nehring, and W. R. Wichser. 1975. Aflatoxin contamination of corn in the field. J. Agric. Food Chem. 23:775-782.

Beckwith, A. C., R. F. Vesonder, and C. Ciegler. 1976. Chemical method investigated for detoxifying aflatoxins in foods and feed. Pages 58-67 in J. V. Rodricks, ed. Mycotoxins and other fungal related food problems. American Chemical Society, Washington, D. C.

Bilay, V I. 1960. Mycotoxicosis of Man and Agricultural Animals. Kiev, USSR. 167 pp.

Brekke, O. L., H. F Conway, A. J Peplinski, C. Vojnovich, and R. A. Anderson. 1979. Deodorization of ammoniated corn by drying and other methods. Trans. Am. Soc. Agric. Eng. 22:421-424.

Busby, W. F. Jr, and G. N. Wogan. 1984. Aflatoxins. Pages 945-1136 in C. D. Searle, ed. Chemical carcinogens. American Chemical Society, Washington, D. C.

Butler, W. H. 1974. Aflatoxin. Pages 1-28 in I. F. H. Purchase ed. Mycotoxins. Elsevier Science Publishers, New York, NY.

Chelkowski, J., K. Szibiotko, P. Golinski, M. Buchowski, B. Godlewska, W. M. Radonyska, and M. Wiewiorowska. 1982. Mycotoxins in cereal grain. Part 5. Changes in cereal grain biological value after ammoniation and mycotoxins inactivation. Die Nahrung. 25:631-637.

Coker, R. D., K. Jewers, N. R. Jones, J. Nabney and D. H. Watson. 1983. Decontaminating aflatoxin-containing agricultural products. British Patent 2108365, London, UK. 4 pp.

Coker, R. D., K. Jewers, and B. D. Jones. 1985. The destruction of aflatoxin by ammonia: Practical possibilities. Trop. Sci. 25:139-154.

Crosslin, G. P., L. M. Seitz, K. C. Behnke, P. E. Sanford, and C. W. Deyoe. 1979. Aflatoxin detoxification by thermal processing in combination with chemical treatment. Poult. Sci. 58:1047. (Abstr.)

Dollear, F. G., F. E. Mann, L. P. Codifier, H. K. Gardner, Jr, S. P. Koltun, and H. L. E. Vix. 1968. Elimination of aflatoxin from peanut meal. J. Am. Oil Chem. Soc. 45:862-865.

Dollear, F. G. 1969. Detoxification of aflatoxins in foods and feeds. Pages 359-391 in L. A. Goldblatt ed. Aflatoxin-scientific background, control, and implications. Academic Press, New York, NY.

Dorner, J. W. 1990. Methods of analysis and confirmation of aflatoxins in commodities. Pages 12-19 in A perspective on aflatoxin in field crops and animal food products in the United States. Agriculture Research Service, Washington, D.C.

Ferrando, R., A. L. Parodi, N. Henry, A. L. N'diaye, ,C. Fourlon, and P. DeLort-Laval. 1975. Influence d'un traitement par l'ammoniaque du tourteau d'arachide contamine par A. flavus sur son effeicaite alimentaire chez le caneton. Ann. Nutr. Aliment. 20:61-66.

Food and Drug Administration. 1974. Aflatoxins in shelled peanuts and peanut products used as human foods: Proposed tolerance. Fed. Reg. 39:42748-41751.

Fowler, R. G. 1979. Aflatoxin: Ammonia is helping scientists bag the problem of contaminated cottonseed. Prog. Agric. Arizona. 30:3-7.

Goldblatt, L. A. 1971. Control and removal of aflatoxin. J. Am. Oil Chem. Soc. 48:605-610.

Goldblatt, L. A., and F. G. Dollear. 1977. Review of prevention, elimination, and detoxification of aflatoxins. Arch. Inst. Pasteur Tunis. 54:411-422.

Guthrie, L. B., C. Hammond, and T. Lawson. 1981. Detoxification of aflatoxin corn on a Georgia farm with anhydrous ammonia. J. Dairy Sci. 64(Suppl.1):218.

Hesseltine, C. W. 1979. Introduction, definition, and history of mycocoxins of importance to animal production. Pages 3-19 in Interactions of mycocoxins in animal production. National Academy of Sciences, Washington, D.C.

Hsieh, D. P. S. 1979. Basic metabolic effects of mycotoxins. Pages 43-55 in Interactions of Mycotoxins in Animal Production. National Academy of Sciences, Washington, D.C.

Jemmali, M. 1980. Decontamination and detoxification of mycotoxins. Pure Appl. Chem. 52:175-181.

Jemmali, M. 1983. Decontamination of mycotoxins. Pages 143-150 in Proc. int. symp. mycotoxins. National Research Centre, Cairo, Egypt.

Keppler, J. G., and H. deLongh. 1964. Aflatoxin - Occurence and composition. Food Cosmet. Toxicol. 2:675.

Keyl, A. C., and W. P. Norred. 1978. Utilization of aflatoxin contaminated feed. Am. Dairy Sci. Assoc./Am. Soc. Anim. Sci. East Lansing, MI.

Kiermeier, F., and L. Ruffer. 1974. Veranderung von aflatoxin B_1 in alkalischer losung. Z. Lebensm. Unters. Forsch. 155:129-141.

Kobbe, B., D. P. H. Hseih, and W. L. Dunkley. 1979. Aflatoxin in corn - What is it? How does it get there? Dairyman. 59:A6-A7.

Koltun, S. P., E. T. Rayner, J. I. Wadsworth, and H. K. Gardner, Jr. 1979. Inactivation of aflatoxins in cottonseed meal by ammoniation. I. Reaction studies. J. Am. Oil Chem. Soc. 56:803-806.

Lawlor, T. E., S. R. Haworth, E. Zeiger, D. L. Park, and L. S. Lee. 1985. Mutagenic potential of ammonia-related aflatoxin reaction products in cottonseed meal. J. Am. Oil Chem. Soc. 62:1136-1138.

Lee, L. S., D. L. Park, S. R. Haworth, S. P. Koltun, and A. Ceigler. 1983. Mutagenic potential of aflatoxin related decontamination by products resulting from ammoniation of cottonseed meal. J. Am. Oil Chem. Soc. 60:696. (Abstr.).

Lee, L. S., J. J. Dunn, A. J. DeLucca, and A. Ciegler. 1981. Role of lactone ring of aflatoxin B_1 in toxicity and mutagenicity. Experientia 37:16-17.

Lillehoj, E. B. 1979. Natural occurrence of mycotoxins in feeds: pitfalls in determination. Pages 139-153 in Interactions of mycotoxins in animal production. National Academy of Sciences, Washington, D.C.

Mann, G. E., H. K. Gardner, Jr, A. N. Booth, and M. R. Gumbmann. 1971. Aflatoxin inactivation: Chemical and biological properties of ammonia and methylamine treated cottonseed meal. J. Agric. Food Chem. 19:1155-1158.

Miyake, M. and M. Saito. 1965. Liver injury and liver tumors induced by toxin of Penicillium islandicum Sopp growing on yellowed rice. Pages 133-146 in Mycotoxins in foodstuffs. MIT Press, Cambridge, MA.

Muller, H. M. 1984. A survey of methods of decontaminating mycotoxins. II. Chemical methods and reaction with components of feedstuffs. Anim. Res. Dev. 19:7-37.

Norred, W. P. 1981. Excretion and distribution of ammoniated ^{14}C-labeled aflatoxin B1 containing corn. Fed. Proc. 41:694.

Park, D. L. and L. S. Lee. 1990. New perspective on the ammonia treatment for decontamination of aflatoxins. Pages 127-137 in A perspective on aflatoxin in field crops and animal food products in the United States. Agricultural Research Service, Washington, D.C.

Park, D. L., L. S. Lee, and S. P. Koltun. 1984. Distribution of aflatoxin in corn meal. J. Am. Oil Chem. Soc. 61:1071-1074.

Park, D. L. and A. E. Pohland. 1986. A rationale for the Control of aflatoxin in animal feeds. Pages 373-382 in P. Steyn and R. Vleggaar eds. Mycotoxins and phytotoxins. Elsevier Science Publishers, Amsterdam, The Netherlands.

Santi, E., A. Pietri, and G. Piva. 1982. Detoxification of aflatoxin B_1 in peanut meal by urea treatment. Ann. Fac. Agric. Catholic Univ. Sacred Heart (Piacenza) 22:299-302.

Schroeder, T., J. Luthy, P. M. Sagelsdorff, U. M. Zweigel, U. Friederich, and C. Schlatter. 1981. Biological activity of (^{14}C)-aflatoxin B_1 degradation products obtained during ammonia detoxification. Mitt. Lebensmittelunters. Hyg. 72:62-70.

Schuller, P. L., H. P. van Egmond, and L. Stoloff. 1983. Limits and regulations on mycotoxins. Pages 111-129 in Proc. int. symp. mycotoxins. National Research Centre, Cairo, Egypt.

Southern, L. L., and A. J. Clawson. 1979. Ammoniation of aflatoxin contaminated corn in diets for growing rats. Am. J. Clin. Nutr. 32:R28.

Southern L. L., and A. J. Clawson. 1980. Ammoniation of aflatoxin contaminated corn with aflatoxin and its effects on growing rats. J. Anim. Sci. 50:459-466.

Stahr, H. M. 1991. Analytical methods in toxicology. John Wiley and Sons, Inc., New York. 213 pp.

Thiesen, J. 1977. Detoxification of aflatoxins in groundnut meal. Anim. Feed Sci. Technol. 2:67-75.

Tuite, J. 1979. Field and storage conditions for the production of mycotoxins and geographic distribution of some mycotoxin problems in the United States. Pages 19-39 in Interactions of mycotoxins in animal production. National Academy of Sciences, Washington, D.C.

Van der Merwe, K. J., P. S. Steyn, and L. Fourie. 1965. Mycotoxins. Part II. The constitution of ochratoxin A, B, and C, metabolites of Aspergillus ochraceus. Wilh. J. Chem. Soc. 1965:7083-7088.

Van Egmond, H. P. 1987. Current situation on regulations for mycotoxins: Overview of tolerances and status of standard method of sampling and analysis. Report to FAO (Project No. 388609), FAO, Rome, Italy. 192 pp.

Vesonder, R. F., A. C. Beckwith, A. Ciegler, and R. J. Dimier. 1975. Ammonium hydroxide treatment of aflatoxin B_1: Some chemical characteristics and biological effects. J. Agric. Food Chem. 23:242-248.

ACKNOWLEDGEMENTS

It gives me an immense pleasure to express my profound sense of gratitude to my major professor Dr. G.D. Osweiler for his expertise help, guidance and keen interest in the project.

Special thanks are also due to my advisory committee members: Dr. P.A. Murphy and Dr. M.G. Stahr for their interest and help during this study.

I also express my thanks to Dr. S.T. Witte, Dr. P.J. Imerman, Mr. Shantilal Parmar, and Miss Fangli Wang for their help and advice.

I would also like to express special thanks to my parents, for their loving support and encouragement throughout my study in ISU.

Finally, I wish to send out of my deepest love and appreciation to my wife, Ying Chen, for her unending encouragement, support and understanding.