FOOD CHEMICAL CONTAMINANTS

Analysis of Aflatoxins in Poultry and Pig Feeds and Feedstuffs Used in Colombia

ANGEL E. CÉSPEDES and GONZALO J. DIAZ[1]
Universidad Nacional de Colombia, Facultad de Medicina Veterinaria y de Zootecnia, Bogotá, Colombia

Feedstuffs and mixed feeds used for poultry and pig nutrition in Colombia were analyzed for aflatoxins by using a liquid chromatographic technique with a limit of detection of 1 μg/kg for each aflatoxin (B_1, B_2, G_1, and G_2). Samples of grain sorghum, maize, processed soybean, rice meal, cottonseed meal, and poultry and pig feeds, representative of Colombian production for the 1995–1996 harvest, were taken from feed-manufacturing plants in various cities. Aflatoxins were detected in 11 of 45 samples of sorghum, 4 of 33 samples of maize, 8 of 22 samples of rice meal, 15 of 17 samples of cottonseed meal, 1 of 12 samples of other feedstuffs, 12 of 30 samples of poultry feed, and 7 of 16 samples of pig feed. Aflatoxins were not detected in soybean. Only 9 of 58 positive samples contained total aflatoxin levels exceeding maximum tolerable limits in Colombia.

Aflatoxins are a closely related group of heterocyclic metabolites synthesized predominantly by the fungi *Aspergillus flavus* Link and *Aspergillus parasiticus* Speare (1). Aflatoxins were first identified as the causative agent of the severe outbreak of "Turkey X" disease, a toxicosis that killed more than 100 000 turkey poults in England in 1960 (2). They are a major concern as human hepatocarcinogens and are considered to play an important rôle in the high incidence of human hepatocellular carcinoma in certain areas of the world (3). Animal exposure to aflatoxins, particularly to aflatoxin B_1, may result in hepatotoxicosis, mutagenesis, immunosuppression, teratogenesis, or carcinogenesis (4). The incidence and level of aflatoxin contamination in various commodities has been monitored worldwide (5). However, although many surveys of natural occurrence of mycotoxins in several commodities are conducted every year in industrialized countries (6), very little information is generated in developing countries. The lack of adequate laboratories and human and economic resources may be a reason for the lack of information on mycotoxin ocurrence in developing countries.

Molds that produce aflatoxins are more common in warm and humid tropical climates (7); however, little information concerning aflatoxin contamination in Colombian feeds and feedstuff has been gathered to date. The aim of the present study was to investigate the incidence and level of aflatoxin contamination of Colombian feeds and feedstuffs for poultry and pig.

Experimental

Samples

Samples of cereal grains and mixed feeds were taken at feed-manufacturing plants in various Colombian cities, including Cartagena, Guadalajara de Buga, Medellín, Mosquera, and Santafé de Bogotá. Samples were obtained from 5 companies, which commercialize and produce about 95% of the poultry and pig feeds and feedstuffs in Colombia. Sampling and subsampling of raw materials and mixed feed were conducted according to procedures recommended by Campbell et al. (8) and Park and Pohland (9). Raw materials representative of Colombian production for the 1995–1996 harvest were sampled as they arrived at the feed-manufacturing plant. Samples of mixed feed were taken from stored feed. A total of 200 samples were analyzed for aflatoxins: 45 samples of grain sorghum, 33 samples of maize, 25 samples of soybean, 22 samples of rice meal, 17 samples of cottonseed meal, 12 samples of other feedstuffs, 30 samples of poultry feed, and 16 samples of pig feed. Collected samples were stored in paper bags in a cool dry place until they were analyzed, within 1 week of collection.

Analysis

Aflatoxins were analyzed according to the method reported by Trucksess et al. (10), with a few modifications: 50 g ground analytical sample was extracted with 100 mL acetonitrile–H_2O (84 + 16) for 1.0 h by using

Received April 3, 1997. Accepted by AP July 17, 1997.
[1] Permanent address: Apartado Aéreo 76948, Santafé de Bogotá, D.C., Colombia.

a wrist-action shaker at high speed (a 25 g sample was taken in the case of hygroscopic feedstuffs such as rice meal). The extract was filtered through qualitative high-speed filter paper, and ca 5 mL filtered extract was transferred into a 10 mL culture tube. The extract was purified with a multifunctional cleanup column (Mycosep 224 MFC column, Romer Laboratories, Union, MO). The column was pushed slowly into the culture tube until ca 0.5 mL purified extract appeared in the column reservoir. Then 200 μL purified extract was transferred quantitatively to a derivatization vial with Teflon-lined screw cap. To the extract was added 700 μL derivatizing solution (trifluoroacetic acid–glacial acetic acid–H_2O, 2 + 1 + 7) to transform aflatoxins B_1 and G_1 into aflatoxins B_{2a} and G_{2a}, respectively. The vial was closed tightly, shaken on a Vortex mixer for 30 s, and heated in a 65°C water bath for 10 min. After the vial was cooled to room temperature, the contents were filtered and injected into an LC-9A liquid chromatograph (Shimadzu, Kyoto, Japan). Chomatographic conditions were as follows: mobile phase, isocratic H_2O–methanol (60 + 40) at a flow rate of 1.0 mL/min; analytical column, reversed-phase ODS, 25 cm × 4.6 mm id (Supelcosil LC-18, Supelco, Bellefonte, PA); column temperature, 50°C (CTO-6A, column oven; Shimadzu); fluorescence detector (Model RF-535, Shimadzu) excitation wavelength, 350 nm, emission wavelength, 450 nm. The limit of detection was 1.0 μg/kg for individual aflatoxins (B_1, B_2, G_1, and G_2). Figure 1 shows chromatograms of aflatoxin-free commodities, naturally contaminated commodities, and spiked samples.

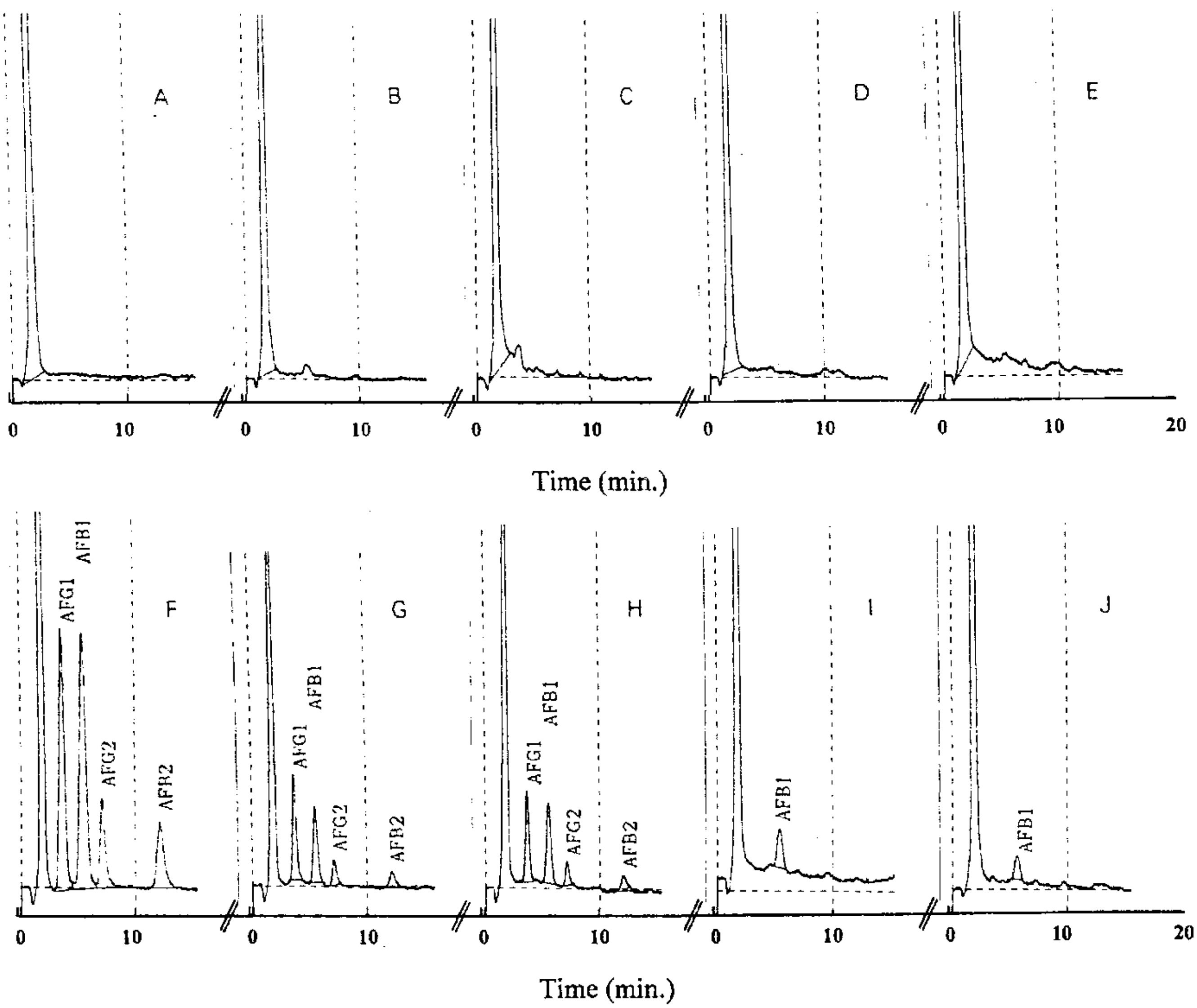

Figure 1. Chromatograms of aflatoxin-free (A) sorghum grain, (B) soybean meal, (C) rice meal, (D) poultry feed, and (E) pig feed; (F) standard aflatoxin mixture equivalent to 65 ng/g total aflatoxins (25:25:7.5:7.5 G_1:B_1:G_2:B_2); (G) grain sorghum spiked with 13 ng/g total aflatoxins (5:5:1.5:1.5 G_1:B_1:G_2:B_2); (H) poultry feed spiked with 13 ng/g total aflatoxins (5:5:1.5:1.5 G_1:B_1:G_2:B_2); (I) pig feed naturally contaminated with 3.2 ng/g aflatoxin B_1; and (J) cottonseed meal naturally contaminated with 1.8 ng/g aflatoxin B_1.

Table 1. Method performance for various substrates

Substrate	Aflatoxin B_1, ng/g substrate	S_T	RSD_T, %	Recovery, %
Sorghum	5	0.21	5.0	82
Sorghum	50	1.46	3.3	88
Soybean meal	5	0.12	2.8	98
Soybean meal	50	0.36	0.8	94
Rice meal	5	0.46	10.2	90
Rice meal	50	0.78	1.8	87
Cottonseed meal	5	0.29	6.5	88
Cottonseed meal	50	1.59	3.3	95
Poultry feed	5	0.51	10.5	98
Poultry feed	50	2.06	4.6	90
Pig feed	5	0.70	14.7	96
Pig feed	50	14.8	27.1	109

(*Caution:* Wear protective clothing, gloves, and eye wear. *See* material safety data sheets for each reagent. Soak all laboratory glassware and plasticware in detergent before washing or disposal. For safe handling of aflatoxins, *see* precautions described in introductory paragraph of Chapter "Natural Poisons" and in section **977.16** (11). Dispose of waste solvents in appropriate manner compatible with applicable environmental rules and regulations).

Method Performance

Method performance was determined for substrates not previously tested by Trucksess et al. (10) by spiking blank samples at 2 levels in triplicate. Results are summarized in Table 1.

Quality control included confirmation of 10 positive samples (taken at random) by thin-layer chromatography (TLC). A 2 mL volume of purified extract (obtained from multifunctional cleanup column) was taken to dryness and dissolved in 100 μL benzene–acetonitrile (98 + 2). Then 20 μL of this solution was spotted on silica gel TLC plates and developed with chloroform–acetone (90 + 10), along with appropriate standards. Aflatoxin spots were visualized under long-wave UV light (11). The identity of aflatoxin B_1 was confirmed also by injecting into the liquid chromatograph the extract of positive samples without previous derivatization (the peak of aflatoxin B_1 dissapears). Aflatoxin standards were obtained from Sigma Chemical Co. (St. Louis, MO). Their authenticity was verified by TLC, and they were quantitated on the basis of absorptions of individual aflatoxins in the UV range taken with recording spectrophotometer (Shimadzu Model 160A UV-Vis spectrophotometer: 11).

Results and Discussion

Aflatoxins were found in all commodities analyzed, except soybean. Aflatoxin B_1 was the major contaminant, with an overall incidence of 29% (58 of 200 samples). The incidence was higher in complete feeds (41.3%) than in feedstuffs (25.3%). Levels of aflatoxin B_1 ranged from 1.0 to 66.1 μg/kg, with a mean of 9.9 μg/kg. The incidence and levels of aflatoxin B_2 were much lower than those for aflatoxin B_1. Only 15 samples (7.5%) had aflatoxin B_2. Levels ranged from 1.1 to 10.4 μg/kg, with a mean value of 2.5 μg/kg. Aflatoxin G_1 was found in only 2 samples (1%), and aflatoxin G_2 was detected in only one (0.5%). All samples containing aflatoxins B_2, G_1, or G_2 also had aflatoxin B_1.

Among feedstuffs, the highest incidence of aflatoxin B_1 was in cottonseed meal, with almost all samples (15 of 17) containing detectable levels (Table 2). However, the highest concentration in a sample was only 11.4 μg/kg, which is below the 20 μg/kg level accepted

Table 2. Incidence and levels of aflatoxin B_1 in major feedstuffs and complete feeds used for pig and poultry nutrition in Colombia

Sample	No. analyzed	No. of positive samples	Incidence, %	Mean, μg/kg	Range, μg/kg
		Feedstuff			
Sorghum	45	11	24.4	10.6	1.4–42.6
Maize	33	4	12.1	20.7	3.9–66.1
Soybean	25	0	0	—	—
Rice meal	22	8	36.4	20.5	1.0–52.8
Cottonseed meal	17	15	88.2	5.3	1.5–11.4
Others[a]	12	1	8.3	2.1	—
Mean			25.3	11.4	1.0–66.1
		Mixed feeds			
Poultry feed	30	12	40.0	6.9	1.5–23.2
Pig feed	16	7	43.8	6.8	1.7–12.3
Mean			41.3	6.9	1.5–23.2

[a] Wheat by-products, cassava meal, and palm meal.

in most countries (12). The second highest incidence of aflatoxin B_1 was in rice meal, with 36.4% of samples (8 of 22) containing detectable levels. For positive samples, the mean concentration of aflatoxin B_1 was 20.5 μg/kg, which is just above the maximum tolerable level accepted in most countries (12). Of the 8 samples of rice meal containing aflatoxin B_1, 3 had levels exceeding 20 μg/kg: 33.4, 50.2, and 52.8 μg/kg. Among samples of sorghum grain, 11 of 45 were contaminated with aflatoxin B_1 (24.4%), but only one had a concentration exceeding 20 μg/kg (42.6 μg/kg). This sample was the only one that contained all 4 naturally occurring aflatoxins (B_1, B_2, G_1, and G_2, 54.8 μg/kg total). Only 4 of 33 maize samples contained detectable amounts of aflatoxin B_1. However, one sample had 66.1 μg/kg aflatoxin B_1; the other 3 contained 3.9, 4.0, and 8.8 μg/kg aflatoxin B_1. The maize sample with aflatoxin B_1 at 66.1 μg/kg also had aflatoxins B_2 (10.4 μg/kg) and G_1 (2.5 μg/kg). None of the 25 samples of processed soybean (soybean meal and full-fat soybean) had detectable levels of aflatoxins.

The incidence of aflatoxin B_1 in the present study (29%) is similar to that found in Brazil by Baldissera et al. (13). These authors reported an incidence of aflatoxin contamination of 24.86% in 519 samples analyzed during the period 1987–1991. However, the levels of aflatoxins reported in Brazil, ranging from 4 to 1906 μg/kg, were much higher than those found in the present study.

The incidence of aflatoxin B_2 was much lower than for aflatoxin B_1 (Table 3). Only 13 of 154 samples of feedstuffs contained aflatoxin B_2 at levels ranging from 1.1 to 10.4 μg/kg. The highest incidence of aflatoxin B_2 was in cottonseed meal, with 35.3% of samples (6 out of 17) containing detectable levels: however, for positive samples, the mean concentration was very low (2.6 μg/kg), and levels ranged from 1.1 to 10.4 μg/kg. The highest level of aflatoxin B_2 (10.4 μg/kg) was found in a maize that also contained aflatoxins B_1 (66.1 μg/kg) and G_1 (2.5 μg/kg).

The incidence of aflatoxin B_1 in mixed feeds was higher than in feedstuffs (Table 2), suggesting that the mixing of different ingredients in a complete feed increases the likelihood of finding aflatoxins. The overall incidence of aflatoxin B_1 in complete feeds was 41.3%, with very similar values for poultry feed (40.0%) and pig feed (43.8%). The mean aflatoxin B_1 contents in positive samples were almost identical for poultry and pig feeds, 6.9 and 6.8 μg/kg, respectively. Even though the incidence of aflatoxin contamination in mixed feeds was relatively high, only 2 samples contained more than 20 μg/kg. Both were poultry feed, with 22.1 and 23.2 μg/kg aflatoxin B_1. Only 2 of 46 samples of mixed feeds analyzed contained aflatoxin B_2 at very low levels (1.1 and 2.0 μg/kg; Table 3).

Conclusions

The survey indicates that aflatoxins are present in about 25% of feedstuffs used for poultry and pig nutrition in Colombia. Aflatoxin occurrence is higher in mixed feeds than in feedstuffs. However, few of the samples exceed the maximum tolerable levels specified by Colombian regulations. To keep aflatoxin contamination at the lower possible levels, surveillance of various commodities and mixed feeds should continue. The tolerance level of total aflatoxins in Colombia is 10 μg/kg for groundnuts, 20 μg/kg for poultry feeds, and 30 μg/kg for cereals (sorghum and millet) (14). The present survey indicates that only 9 of 200 samples analyzed exceed tolerable levels: one maize sample

Table 3. Incidence and levels of aflatoxin B_2 in major feedstuffs and complete feeds used for pig and poultry nutrition in Colombia

Sample	No. analyzed	No. of positive samples	Incidence, %	Mean, μg/kg	Range, μg/kg
		Feedstuff			
Sorghum	45	2	4.4	2.4	1.1–3.7
Maize	33	1	3.0	10.4	—
Soybean	25	0	0	—	—
Rice meal	22	4	18.2	2.4	1.1–3.3
Cottonseed meal	17	6	35.3	1.6	1.1–2.0
Others[a]	12	0	0	—	—
Mean			8.4	2.6	1.1–10.4
		Mixed feeds			
Poultry feed	30	1	3.3	2.0	—
Pig feed	16	1	6.3	1.1	—
Mean	46	2	4.3	1.6	1.1–2.0

[a] Wheat-by products, cassava meal, palm meal.

(79.0 μg/kg total aflatoxins), one sorghum sample (54.8 μg/kg total aflatoxins), 3 rice meal samples (35.5, 53.1 and 56.1 μg/kg total aflatoxins), 2 cottonseed meal samples (10.7 and 13.1 μg/kg total aflatoxins), and 2 poultry feed samples (22.1 and 25.2 μg/kg total aflatoxins). Because of the lack of information, surveys on the incidence of aflatoxins and other mycotoxins in human foods should be conducted in Colombia. Surveys to determine the incidence and levels of mycotoxins other than aflatoxin in Colombian foods, feeds and feedstuffs also are needed.

Acknowledgment

We thank the Committee for Research and Scientific Development of the National University of Colombia for funding (research grant No. 811040).

References

(1) Ellis, W.O., Smith, J.P., Simpson, B.K., & Oldham, J.H. (1991) *Crit. Rev. Food Sci. Nut.* **30**, 403–439

(2) Asplin, F.D., & Carnaghan, R.B.A. (1961) *Vet. Rec.* **73**, 1215–1219

(3) Choy, W.N. (1993) *Mutat. Res.* **296**, 181–198

(4) Lesson, S., Diaz, G., & Summers, J. (1995) *Poultry Metabolic Disorders and Mycotoxins*. University Books, Guelph, ON, Canada

(5) Jelinek, C.F., Pohland, A.E., & Wood, G.E. (1989) *J. Assoc. Off. Anal. Chem.* **72**, 223–230

(6) Scott, P.M. (1993) *J. AOAC. Int.* **76**, 112–119

(7) Reddy, C.V. (1992) *Misset-World Poult.* **8**(6), 19, 21

(8) Campbell, A.D., Whitaker, T.B., Pohland, A.E., Dickens, J.W., & Park, D.L. (1986) *Pure Appl. Chem.* **58**, 305–314

(9) Park, D.L., & Pohland, A.E. (1989) *J. Assoc. Off. Anal. Chem.* **72**, 399–404

(10) Trucksess, M.W., Stack, M.E., Nesheim, S., Albert, R.H., & Romer, T.R. (1994) *J. AOAC Int.* **77**, 1512–1521

(11) *Official Methods of Analysis* (1990) 15th Ed., AOAC, Arlington, VA

(12) Van Egmond, H.P. (1989) *Food Addit. Contam.* **6**, 139–188

(13) Baldissera, M.A., Santurio, J.M., Canto, S.H., Pranke, P.H., Almeida, C.A.A., & Schimidt, C. (1993) *Rev. Inst. Adolfo Lutz* **53**, 5–10

(14) Resnik, S., Costarrica, M.L., & Pacin, A. (1995) *Food Control* **6**, 19–28