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Survey of Aflatoxin Concentrations in Wild Bird Seed Purchased in Texas

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ABSTRACT: The use of backyard feeders to attract avian wildlife is a common practice throughout the United States. However, feeding wildlife may create a problem due to aflatoxin, a harmful fungal metabolite, which can affect wildlife that are fed contaminated grain. Our study was initiated to determine if songbirds were being exposed to aflatoxin-contaminated feed throughout Texas. Bags of wild bird seed ($n = 142$) were purchased from grain cooperatives, grocery stores, and pet shops located in the panhandle, central, south, east, and west regions of Texas during spring and summer 1999. Aflatoxin concentrations in bird seed ranged from non-detectable to 2,780 μg/kg. Overall, 17% of samples had aflatoxin concentrations greater than 100 μg/kg, of which 83% contained corn as an ingredient. Retail establishment effects were noted in the southern and western regions of Texas, with average concentrations of aflatoxin greater from bags of bird seed purchased from grain cooperatives, followed by pet shops, then grocery stores. Regional differences in aflatoxin levels were not apparent from bags of seed purchased at pet shops; however, regional differences were noted in aflatoxin levels from seeds obtained at grocery stores and grain cooperatives. Average aflatoxin concentration from seed purchased at grocery stores was greatest in the panhandle region, followed by the remaining regions. Within grain cooperatives, the panhandle, south, and west regions of Texas exhibited higher levels of aflatoxin-contaminated bird seed than cooperatives within the east and central regions of Texas. Granivorous songbirds in Texas are exposed to aflatoxins at backyard feeders, which may be a significant morbidity and mortality factor.

Key words: Aflatoxin, *Aspergillus flavus, Aspergillus parasiticus,* bird seed, Texas, toxicology.

Many urban and suburban landowners extensively use backyard feeders to attract avian wildlife for viewing and photographing. DeGraaf and Payne (1975) estimated that about 20% of all United States households purchase an average of 27.3 kg of birdseed each year. The 1990 population census states that about 53 million households existed in the United States during the 1980's (http://www.census.gov). In addition, results of the 1996 National Survey of Fishing, Hunting, and Wildlife-Associated Recreation suggest that 5.2 million American households regularly feed wild birds (USFWS, 1996). Based on this information, we estimate between 141,960 to 289,380 metric tons of bird seed are distributed annually in the United States. However, supplemental feeding of songbirds, even though well intended, may expose birds to mycotoxins.

Mycotoxins are potent carcinogenic, mutagenic, teratogenic, and immunosuppressive agents, of which aflatoxin is one of the most widely occurring and dangerous (C.A.S.T., 1989). Aflatoxin is a harmful fungal metabolite produced by strains of *Aspergillus flavus* and *Aspergillus parasiticus,* which can potentially affect wildlife that are fed contaminated grain. Any cereal grain is susceptible to the production of aflatoxin; however, factors such as warm temperatures, high relative humidity and moisture level of feed, drought, insect infestation, and damaged grain kernels can enhance the probability of aflatoxin production (Jacques, 1988). Aflatoxin resulting from mold growth may be present at the time of harvest or may occur any time during processing, transport, or storage if conditions are favorable. However, Thompson and Henke (2000) noted that aflatoxin can be produced regardless of type of storage container, time of storage, and climatic conditions.

Because of the deleterious effects of aflatoxin, the U.S. Food and Drug Administration (Washington, D.C.) has estab-

TABLE 1. Locations within five regions of Texas where bags of bird seed were purchased from grocery stores, pet shops, and grain cooperatives during spring and summer 1999.

Geographic regions[a]				
Panhandle	Central	South	East	West
Amarillo	San Angelo	Cotulla	Dallas	Midland
Plainview	Junction	Beeville	Tyler	Fort Stockton
Lubbock	Austin	Refugio	Hillsboro	Monahans
Snyder	Seguin	Corpus Christi	Waco	Pecos
Ballinger	San Antonio	Alice	Lufkin	Alpine
Abilene	Kerrville	Falfurrias	Brenham	El Paso
Wichita Falls	Brownwood	Edenberg	Houston	Del Rio
Dalhart	Fredericksburg	Weslaco	Texarkana	Odessa
Brownfield	Lampassas	Harlingen	Bryan	Fabens
Lamesa	Brady	Laredo	Temple	Kermit

[a] Geographic regions were selected based on gradual climate changes within the state. Panhandle region (i.e., warm and dry climate) was designated as north of an imaginary line from Wichita Falls to the southwest corner of Andrews County. West region (i.e., hot and dry climate) was designated as west of an arc from the southwest corner of Andrews County to Del Rio. South region (i.e., hot and humid climate) was considered south of an imaginary line drawn from Victoria to Del Rio. East region (i.e., warm and humid climate) was designated as the area east of an imaginary line from Victoria to Wichita Falls. The Central region (i.e., intermediate climate) was considered the center portion of the state not previously designated.

lished a limit of 20 μg/kg aflatoxin for various classes of animal feed (Rustom, 1997; Quist et al., 1997). Unfortunately, grain that has been condemned for human or domestic animal consumption typically gets marketed as feed for wildlife (Quist et al., 1997). Fischer et al. (1995) noted that 51% of 39 samples of shelled corn used as bait in the eastern United States for white-tailed deer (*Odocoileus virginianus*) were positive for aflatoxin, with levels reaching 750 μg/kg. However, the exposure of wild birds to aflatoxin-tainted grain is unexplored. Our objectives were to determine if songbirds are being exposed to aflatoxin at feeders in Texas, and if type of retail establishment and geographic region cause differences in the concentration of aflatoxin from bags of bird seed purchased in Texas. We hypothesized that retail establishments (i.e., grocery stores, pet shops, and grain cooperatives) differ in their turnover rates of bird seed, with pet shops having the slowest rate than the other retail sources, and thus, result in greater concentrations of aflatoxin. In addition, because most storage facilities within retail establishments are not environmentally controlled, we further hypothesized that environmental conditions in which grain was stored would differ geographically within Texas, and therefore, result in differing levels of aflatoxin production across the state.

We purchased 142 bags of wild bird seed from 50 locations throughout Texas (Table 1). We divided Texas into 5 regions (i.e., panhandle, west, south, east, and central), based on gradual changes in climate. Panhandle region (i.e., warm and dry climate) was designated as north of an imaginary line from Wichita Falls to the southwest corner of Andrews County. West region (i.e., hot and dry climate) was designated as west of a convex arc from the southwest corner of Andrews County to Del Rio. South region (i.e., hot and humid climate) was considered south of an imaginary line drawn from Victoria to Del Rio. East region (i.e., warm and humid climate) was designated as the area east of an imaginary line from Victoria to Wichita Falls. The Central region (i.e., intermediate climate) was considered the center portion of the state not previously designated. Bags of bird seed were obtained from 10 grocery stores, 10 grain cooperatives, and 10 pet shops within each region. Because pet shops were not prevalent throughout the state, only 42 bags of bird seed were acquired from this type of retail establishment. Within each retail establishment, each bag of wild bird seed was assigned a number and one bag was randomly chosen for purchase. Only bags of mixed bird seed were used for our study because we did not want type of seed as a factor in analysis. Bags of mixed bird seed consisted mainly of milo, millet, and sunflower seeds, and occasionally corn. The majority of seed obtained came from three suppliers (Pennington Bird Seed Company, Madison, Georgia, USA; 3-D, Stephen, Minnesota, USA; and Hill Country Fare, San Antonio, Texas, USA). Seeds from grain cooperatives often were mixed on site. Bag sizes ranged from 11 to 55 kg. Seeds were stored at 21 C and approximately 40% relative humidity until analysis, which typically was within one week of purchase.

Seeds within a bag were thoroughly mixed, ground in a Romer mill (Glen Mills, Inc., Clifton, New Jersey, USA), and two 50-g samples were quantified for levels of aflatoxin using the Aflatest® kit and fluorometer (Series 4, Vicam, Watertown, Massachusetts, USA) method. Fluorometer was recalibrated daily with known standards. Detection limit for aflatoxin by this method is 1.0 μg/kg. Aflatoxin concentrations of duplicate samples had to be within 5% of each other, with additional samples run until this criteria was satisfied. Average aflatoxin concentration of the two samples was calculated and used in subsequent analyses. Distributions of residual errors were tested for normality using the Shapiro-Wilk test (SAS Institute, Inc., 1989). Due to non-normal data, data were ranked and then analyzed by a general linear analysis of variance to test the effects of retail establishment type and geographic region of Texas on average aflatoxin concentrations (SAS Institute, Inc., 1989). If a significant interaction was detected, single variates of the interaction were analyzed separately within each level of the other main effect. Multiple comparisons were made with Tukey's studentized range (HSD) test when significant effects were found (Cochran and Cox, 1957). Statistical significance was inferred at $P \leq 0.05$. Descriptive statistics are presented as the mean ±1 standard error. Non-detectable levels of aflatoxin were considered zero and factored into the mean as such. A predictive model using logistic regression was developed to predict the presence of corn based on the aflatoxin concentration. The amount of variation explained by the model (R^2) was adjusted according to Nagelkerke (1991). The jackknife method was used to decrease the bias of classifying the same data in which the classification measure was derived (SAS Institute, Inc., 1989).

Aflatoxin concentrations in bird seed ranged from 0 to 2,780 μg/kg. Overall, 17% of the 142 samples had aflatoxin concentrations greater than 100 μg/kg, and 83% of those samples contained corn as an ingredient. The logistic regression model could correctly predict if corn should be present or absent 72% of the time; however, the model only could correctly determine the presence of corn 52% of the time. Due to its low rate of accuracy, the predictive equation is not offered. Because an interaction between main effects was noted ($F_{8,141} = 2.38$, $P = 0.02$), separate analyses of main effects were conducted (Table 2). Retail establishment differences only were noted in the southern ($F_{2,27} = 6.86$, $P = 0.004$), and western ($F_{2,23} = 6.33$, $P = 0.006$) regions of Texas, with grain cooperatives having greater average concentrations of aflatoxin than grocery stores (Table 2). Regional differences in aflatoxin concentrations were noted from bags purchased from grocery stores ($F_{4,45} = 4.03$, $P = 0.007$) and grain cooperatives ($F_{4,45} = 4.03$, $P = 0.007$) (Table 2). The panhandle region experienced the greatest average aflatoxin concentrations of bird seed purchased from grocery stores. Within grain cooperatives, the panhandle, south, and west regions of Texas exhibited higher average concentrations of aflatoxin-contaminated bird seed than cooperatives

TABLE 2. Average aflatoxin concentration (μg/kg) of bird seed purchased at grocery stores, pet shops, and grain cooperatives within five geographic regions of Texas.

Geographic region	Retail establishment	*n*	$\bar{x} \pm$ SE	Range
East	Grocery	10	7.9 ± 3.1 Aa[a,b,c]	0–27[d]
	Pet shop	10	73.4 ± 42.4 Aa	0–440
	Co-op	10	9.8 ± 4.6 Ab	0–42
Central	Grocery	10	19.1 ± 14.6 Aa	0–150
	Pet shop	9	75.5 ± 49.6 Aa	0–460
	Co-op	10	7.2 ± 2.6 Ab	0–23
South	Grocery	10	7.5 ± 4.0 Aa	1–39
	Pet shop	8	51.6 ± 18.9 ABa	0–120
	Co-op	10	223.9 ± 65.0 Ba	2–490
Panhandle	Grocery	10	124.1 ± 56.0 Ab	1–590
	Pet shop	9	38.0 ± 12.6 Aa	10–110
	Co-op	10	365.2 ± 269.8 Aa	15–2,780
West	Grocery	10	8.1 ± 3.2 Aa	0–26
	Pet shop	6	34.4 ± 17.4 ABa	2–115
	Co-op	10	44.1 ± 11.7 Ba	8–114

[a] General linear analysis of variance was used to test the effects of retail establishment type and geographic region on levels of aflatoxin. Analysis was conducted on ranked data due to non-normal distribution of residuals. Because an interaction between main effects occurred, separate analyses of main effects were conducted.

[b] Means with the same upper case letter are not different ($P > 0.05$) between retail establishment types within a geographic region.

[c] Means with the same lower case letter are not different ($P > 0.05$) between geographic regions within the same retail establishment type.

[d] Aflatoxin concentrations that were non-detectable are listed as zero.

within the east and central regions of Texas.

Granivorous birds in Texas are being exposed to aflatoxins in commercially-available bird seed used at backyard feeders. This could pose a serious morbidity and mortality factor for songbirds. Unfortunately, few controlled studies concerning aflatoxin on avian wildlife species have been published in peer-reviewed scientific journals. Adverse effects of feeding aflatoxin-contaminated grain have been noted for northern bobwhite (*Colinus virginianus*; Wilson et al., 1978) and turkey (*Meleagris gallopova*) poults (Quist et al., 2000). Egg production in bobwhites was halted, reductions in serum proteins, lipids, and calcium, and increases in alkaline phosphatase, creatine phosphokinase, glutamic oxaloacetic transaminase, liver necrosis, bile duct hyperplasia, Kuppfer cell hyperplasia, and heterophil and lymphocyte cell infiltration were observed at aflatoxin levels of 2,000 and 4,000 μg/kg (Stewart, 1985). Similar undesirable physiologic changes were noted in turkey poults fed aflatoxin levels > 100 μg/kg (Quist et al., 2000). Aflatoxin-induced mortality was observed in bobwhites and ringneck pheasants (*Phasianus colchicus*) at aflatoxin concentrations of 125 μg/kg (Huff et al., 1992; Ruff et al., 1992). In addition, Perez et al. (2001) noted that white-winged doves (*Zenaida asiatica*) and northern bobwhites did not differentiate between aflatoxin-contaminated and non-contaminated feed, while green jays (*Cyanocorax yncas*) selected against aflatoxin-tainted feed. Because songbirds are being exposed to aflatoxin at potentially toxic levels and because some bird species cannot select against aflatoxin, the acute and chronic effects of consuming aflatoxin-contaminated grain must be determined for songbirds. Until then, it would be prudent for consumers to avoid bags of bird seed that contain corn.

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