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SHORT COMMUNICATIONS

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Detection of Aflatoxin-contaminated Grain by Three Granivorous Bird Species

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ABSTRACT: Supplemental feeding of game species and the use of backyard feeders to attract avian wildlife are common practices throughout the United States. However, these activities may expose wildlife to aflatoxins. We tested the hypothesis that wild birds would avoid consuming aflatoxin-contaminated feed. Individual northern bobwhites (*Colinus virginianus*), white-winged doves (*Zenaida asiatica*), and green jays (*Cyanocorax yncas*) were presented with feeders that had four compartments, which contained milo that was contaminated with aflatoxin levels of 0, 100, 500, and 1,000 μg/kg, respectively. Feed remaining was weighed at 6, 12, 18, 24, 36, 48, 60, and 72 hr after the initiation of the trial. White-winged doves and northern bobwhites did not avoid contaminated feed. However, green jays selected against aflatoxin-tainted grain. Because white-winged doves and northern bobwhites did not avoid contaminated feed, the risk of exposure to this potentially hazardous toxin exists for these species.

Key words: Aflatoxin, *Aspergillus flavus, Aspergillus parasiticus, Colinus virginianus, Cyanocorax yncas,* green jay, northern bobwhite, toxicology, white-winged dove, *Zenaida asiatica.*

Supplemental feeding of wildlife has become popular in the USA. Feeders using corn and mixtures of corn and commercial protein pellets are increasingly being used for deer management (Kozicky, 1997), and are exploited by "nontarget" granivorous birds. Additionally, many urban and suburban residents maintain backyard feeders, making grain available to a wide range of avifauna including both resident and migratory species. Although there are no estimates of how many tons of grain are used to feed white-tailed deer (*Odocoileus virginianus*), DeGraaf and Payne (1975) estimated that about 20% of all U.S. households purchase an average of 27.3 kg of birdseed per yr. Supplemental feeding, however well intended, may present an insidious problem for wildlife in the form of mycotoxins.

Mycotoxins are toxic metabolites produced by fungi (Merck Veterinary Manual, 1986). Aflatoxin is one of the most widely occurring and dangerous mycotoxins (C.A.S.T., 1989) that is produced by toxigenic strains of *Aspergillus flavus* and *Aspergillus parasiticus.* Aflatoxin is found in a wide variety of substrates including feed pellets, fodder, pasture, hay, and cereal grains. Consequently, feeding wildlife with highly contaminated grain may increase their risk of morbidity and mortality and potentially mitigate any realized benefit from supplemental feeding.

Although the effects of aflatoxin exposure have been documented for various domesticated animal species, only a few studies have examined the impact of this toxin on avian wildlife. Pen studies have shown that aflatoxin can cause depressed immune function, liver dysfunction, coagulation abnormalities, and death for turkey (*Meleagris gallopova*) poults (Quist et al., 2000), northern bobwhites (*Colinus virginianus*; Wilson et al., 1978; Stewart, 1985), and ring-necked pheasants (*Phasianus colchicus*; Huff et al., 1992; Ruff et al., 1992). Based on these studies, it is clear that aflatoxins can pose a serious threat to granivorous avifauna. However, it is unknown whether birds can detect aflatoxin in their forage, and given a choice, select for noncontaminated feed. The objective of this study was to determine if three species of granivorous birds, representing each of three major bird groups (Galliformes, Columbiformes, and Passeriformes), will avoid ingesting aflatoxin-contaminated grain.

Wild-caught northern bobwhites (n = 24), white-winged doves (*Zenaida asiatica*; n = 24), and green jays (*Cyanocorax yncas*; n = 20) were obtained using funnel traps during October 1998 to January 1999 in the vicinity of Kingsville (Texas, USA; 27°31′N, 97°52′W) and placed individually in cages at the Texas A&M University-Kingsville aviary. Birds were captured and handled in accordance with Texas Parks and Wildlife Department (Austin, Texas) Scientific Permits Nos. SPR-0498-949 and SPR-0993-636 and U.S. Fish and Wildlife Service (Albuquerque, New Mexico, USA) Scientific Permit Nos. MB000430-0 and MB827294-1, respectively. This study was approved by Texas A&M University-Kingsville Animal Care and Use Committee (#1-97-39). Each bird was used for one trial only and then released at the site of its capture.

Birds were allowed a 2-wk acclimation period before beginning trials. Aflatoxin-free food and water were given ad libitum during that time. Aflatoxin (*A. flavus*) was obtained from Sigma Chemical (St. Louis, Missouri, USA) and mixed with milo according to the methods of West et al. (1973) and Quist et al. (1997).

Aflatoxin was added to the milo diet at concentrations of 0 μg/kg, 100 μg/kg, 500 μg/kg, and 1,000 μg/kg. Aflatoxin concentrations in our study were chosen because they are within the range of concentrations that have been found in contaminated store-bought birdseed S.E. (Henke, unpubl. data). The concentration of aflatoxin in the final ration was determined using the Aflatest kit and fluorometer (Series 4, Vicam, Watertown, Massachusetts, USA) for each batch of ration prepared. Detection limit for aflatoxin by this method is 1.0 μg/kg. Mean aflatoxin concentration among batches was 1.6 ± 0.4 μg/kg, 113 ± 9 μg/kg, 520 ± 34 μg/kg, and 1,047 ± 37 μg/kg for 0 μg/kg, 100 μg/kg, 500 μg/kg, and 1,000 μg/kg rations, respectively. Preference trials were conducted using cafeteria style trays containing four compartments with separate spill traps. Trays were built to minimize spillage and spilled food was returned to its respective compartment. A total of 150 g of each diet was placed into a randomly selected compartment at the beginning of the trial. Amount of feed remaining in each compartment was weighed at 6, 12, 18, 24, 36, 48, 60, and 72 hrs after initiation of the trial. Assignment of diets to compartments was rerandomized at each weighing interval to avoid the bias of birds that selected food from the same compartment. Program RODGERS (Krebs, 1989: 600–602) was used to calculate the area under the plotted feed consumption versus time curve, which was then standardized to a maximum of 1.0 (diet preference index) for each diet by each bird. The area beneath the feed consumption curves accounts for the order, rate, and total amount of each diet eaten and is the most appropriate measure of preference for cafeteria-type experiments (Krebs, 1989: 404).

We used a completely randomized design for data analysis. Distributions of residual errors were tested for normality using the Shapiro-Wilk test (SAS Institute, Inc., 1989). Homogeneity of variances among treatments were evaluated with the Bartlett's test (Steel and Torrie, 1980). Because assumptions of parametric tests were satisfied, analysis of variance (SAS Institute, Inc., 1989) was used to test the effect of aflatoxin concentration on the preference indices. Multiple comparisons were made using Tukey's procedure when a significant F-test occurred (Ott, 1993). Tests were considered significant at $P \leq 0.05$.

Northern bobwhites and white-winged doves did not avoid aflatoxin-contaminated feed ($F_{3,92} = 1.13$, $P = 0.34$, $r^2 = 0.74$; $F_{3,92} = 0.47$, $P = 0.70$, $r^2 = 0.68$, respectively). Average Rodgers' indices for these species were similar between aflatoxin-

TABLE 1. Average Rodgers' index of preference for 24 northern bobwhites, 24 white-winged doves, and 20 green jays that were fed milo contaminated with three concentrations of aflatoxin and a control diet over a 3 day period.

Aflatoxin concentration	Rodgers' index of preference					
	Bobwhites		White-winged doves		Green jays	
	x̄	SE	x̄	SE	x̄	SE
0 μg/kg	0.507 A[a]	0.03	0.487 A	0.03	0.862 A	0.04
100 μg/kg	0.514 A	0.04	0.475 A	0.05	0.515 B	0.06
500 μg/kg	0.512 A	0.03	0.515 A	0.06	0.553 B	0.05
1,000 μg/kg	0.506 A	0.05	0.508 A	0.03	0.497 B	0.06

[a] Means with the same letter within a species are not different ($P > 0.05$).

tainted diets and ranged from 0.506 ± 0.05 (1,000 μg/kg) to 0.514 ± 0.04 (100 μg/kg) for northern bobwhites and from 0.475 ± 0.05 (100 μg/kg) to 0.515 ± 0.06 (500 μg/kg) for white-winged doves (Table 1). Both of these species fed indiscriminately from the free choice feeders regardless of aflatoxin level (Fig. 1A, 1B). However, green jays selected against ($F_{3,84} = 9.51$; $P = 0.0001$, $r^2 = 0.54$) aflatoxin-tainted grain (Fig. 1C). Average Rodgers' index for green jays was greatest for the diet containing 0 μg/kg aflatoxin than for the other diets (Table 1). However, differences in feed consumption between the aflatoxin-tainted diets were not noted for green jays (Table 1).

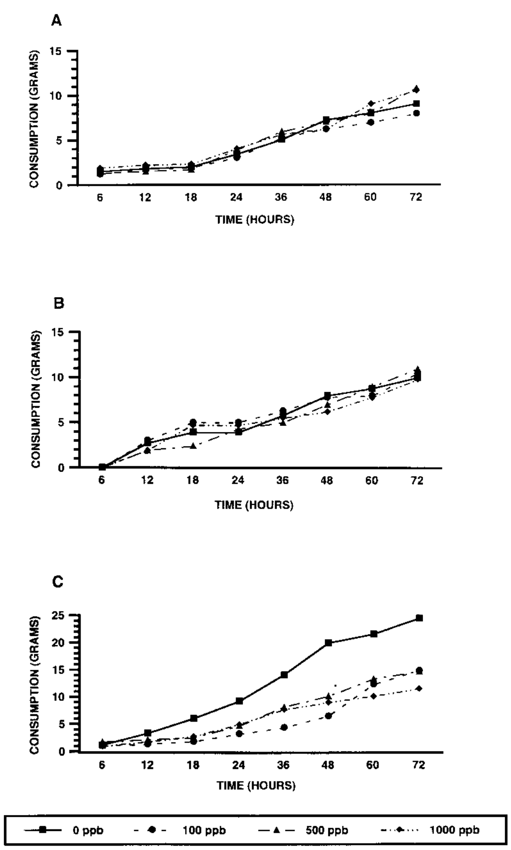

FIGURE 1. Average cumulative food consumption of 24 white-winged doves (A), 24 northern bobwhites (B), and 20 green jays (C) over 3 days with three levels of aflatoxin-contaminated grain and a control.

Although green jays selected against aflatoxin-tainted grain, all green jays consumed some grain that contained aflatoxin. Interestingly, by 72 hr green jays consumed as much aflatoxin-tainted grain as did quail and doves. Therefore, even though green jays selected against grain that contained aflatoxin, consumption of aflatoxin/unit body weight was similar between all species during our study.

It is clear that bird species used in this study differ in their ability to detect and avoid aflatoxin-contaminated feed. Northern bobwhites and white-winged doves did not avoid contaminated feed, thereby increasing their exposure to this toxin. Since LD_{50}'s are unknown for these species, we can not be certain whether or not ingestion of aflatoxin results in increased mortality. However, increased morbidity has been documented in northern bobwhites in controlled pen studies (Wilson et al., 1978; Stewart, 1985; Berthelot, 1996).

Based on our findings, additional research is needed to determine LD_{50}'s for species examined in our study as well as a variety of other species that are routinely exposed to contaminated grain. This is necessary to determine if ingestion of contaminated grain ultimately results in increased morbidity and mortality, particularly since the Texas State Feed and Fertilizer Control Service (Austin, Texas, USA) recommends aflatoxin concentrations <50 μg/kg for wildlife feed (http://info.sos.state.tx.us/pub/plsql/readtac$ext.ViewTAC?tac_view=3&ti=4&pt=3) even though the effects of aflatoxin-tainted grain on wild birds is largely unknown. Such research is needed before safe levels of aflatoxin exposure can be recommended.

Until LD_{50} limits are clearly established for wild granivorous birds, we recommend that individuals involved in supplemental feeding programs make every effort to reduce aflatoxin exposure of granivorous birds. These efforts include purchasing high quality feed, routine testing of feed for aflatoxin, keeping grain dry, and rapid rotation of stored feed (Thompson and Henke, 2000).

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