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AFLATOXIN PRODUCTION IN SUPPLEMENTAL FEEDERS PROVIDED FOR NORTHERN BOBWHITE IN TEXAS AND OKLAHOMA

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ABSTRACT: Mycotoxins are toxic metabolites produced by various species of fungi. Aflatoxin (AF), a particular type of mycotoxin, can negatively impact many wildlife species in the laboratory; however, the magnitude of the problem in the field environment is unclear. Wild birds generally consume a combination of native foods and agricultural grains. A common practice in which birds, such as northern bobwhite (*Colinus virginianus*), contact stored agricultural grain is through supplemental feeding. This feeding practice may promote the production of AF. The objectives of this study were to (1) examine AF production in supplemental feeders and (2) examine the relationship between weather and AF production in supplemental feeders. Samples were collected from supplemental feeders from November through February of 1996–97 and 1997–98. Mean monthly AF concentration of samples from feeders ranged from 0.57 ± 2.86 to 15.47 ± 14.69 ppb. Aflatoxin concentration in supplemental feeders increased from pre-sample to one month after filling the feeders each year. AF production in supplemental feeders was highly variable among months with no real temporal pattern between years. Instead, AF production was related to the highly variable relative humidity of the study area which influences moisture content of grain. Average relative humidity can be used to predict AF production.

Key words: Aflatoxin, *Colinus virginianus*, northern bobwhite, supplemental feeders, weather.

INTRODUCTION

Mycotoxins are toxic metabolites produced by various species of fungi, such as *Aspergillus* spp., *Fusarium* spp., and *Penicillium* spp. (Smith and Moss, 1985). Fumonisin, T-2 toxin, vomitoxin, ochratoxin, and aflatoxin (AF) are a few examples of mycotoxins. These toxins are a large problem in the domestic animal industry where they can cause extensive economic losses (Jones et al., 1996). A wide variety of animals, including fish, rodents, waterfowl, poultry, swine, and cattle can be affected by AF (Wogan, 1966; Robinson et al., 1982; Smith and Moss, 1985; Stewart, 1985; Higgins et al., 1992). Aflatoxin consumption can cause acute hepatitis, hemorrhagic disease, inhibition of protein synthesis, tumor formation, decreased body weight, reduced fertility, decreased resistance to disease, and mortality (Chang and Hamilton, 1979; Smith and Moss, 1985; Ruff et al., 1992; Jones et al., 1996). Aflatoxin also impacts wildlife populations (Robinson et al., 1982; Roffe et al., 1989; Higgins et al., 1992). The significance of the problem for wildlife, however, is largely unknown.

Data concerning impacts of aflatoxin on wildlife in the field are limited. Exposure of agricultural grains to moisture and other environmental conditions present in fields can lead to significant production of AF. Corn left in the field after harvest contained up to 5,000 ppb and 1,210 ppm AF in Mississippi (Couvillion et al., 1991) and Florida (Stewart, 1985), respectively. Comparison of these levels to those used in laboratory experiments suggests many possible detrimental impacts on wildlife (Ruff et al., 1992). Indeed, extensive waterfowl die-offs occurred in two separate areas of Texas in the 1977–78 wintering season; approximately 500 geese and 7,000 ducks succumbed to aflatoxicosis (Robinson et al., 1982) after consuming contaminated grain.

Wildlife may also encounter AF contaminated grain because of supplemental feeding practices. Providing supplemental feed for wildlife, especially northern bob-

white, during winter months and drought is a common management practice (Guthery, 1986). Most feeders have open feeder ports that allow direct access of moisture to grain within the feeder. *Aspergillus* requires temperatures between 6 and 46 C and a relative humidity between 70 and 90%. *Aspergillus* spp. can grow with lower relative humidity if the moisture content of the substrate is between 12 and 17% (Salunkhe et al., 1987). These environmental conditions may occur throughout the year in the Rolling Plains of Texas and Oklahoma (U.S.D.A. 1966; 1975). Though fungal growth on grain in feeders is often readily apparent, feeders are rarely cleaned and new feed is often added to that already present in feeders. This practice may promote production of AF in feeders (Smith and Moss, 1985). Consideration of these common practices, the suitable environmental conditions for *Aspergillus*, spp. and observations of fungal growth in supplemental feeders, leads to the inference that supplemental feeders promote the production of aflatoxin and are a source of aflatoxin for northern bobwhite.

It is clear that given suitable conditions during the growing season, grain may contain some level of aflatoxin before it is harvested or placed in a feeder (Smith and Moss, 1985). For instance, 51% of corn used for feeding deer in North and South Carolina contained AF with concentrations as high as 750 ppb (Fischer et al., 1995). No data concerning how aflatoxin production in grain may be increased by the practice of using supplemental quail feeders is available. The objectives of this study were to (1) examine AF production in supplemental feeders and (2) examine the relationship between weather and AF production in supplemental feeders.

MATERIALS AND METHODS

Study area

The study area was a private ranch located in Wheeler County, Texas and Roger Mills County, Oklahoma (USA; 35°26′N, 100°16′W). Soils are a Pratt-Delwin fine sand on nearly level to sloping terrain (U.S.D.A. 1975). The area is characterized as a sand shinnery oak (*Quercus havardii*)—little bluestem (*Schizachyrium scoparium*) community, although large tracts across the ranch have been planted to weeping lovegrass (*Eragrostis curvula*). Over 200 supplemental feeders are located on the ranch. All feeders are constructed of metal, have a hinged lid and open feeder ports. Feeders are either 57 or 114-kg capacity. The 57-kg capacity feeders measure 0.45 × 0.6 × 0.6 m; 114-kg capacity feeders measure 0.45 × 0.6 × 0.9 m. Feeders are elevated approximately 0.45 m off the ground and are situated near oak motts or Chickasaw plum thickets (*Prunus angustifolia*) to provide cover for northern bobwhites. The standard supplemental feeding practice is to clean out and then fill feeders with a 50/50 mix of corn and sorghum near the end of October each year and keep feeders near full until approximately February the following year. Feeders are not cleaned or emptied of old grain between refilling occasions. Dry winters and humid summers characterize the climate. Most precipitation occurs from April to October. Daily average relative humidity ranged from 20 to 97% from November through February of 1996–97 and 1997–98 (Oklahoma Climatological Survey 1996a, b; 1997a, b, c, d; 1998a, b).

Sample collection

Thirty randomly selected feeders were sampled in November, December, January, and February of 1996–97 and 1997–98. Grain was collected from the same 30 feeders at 25 to 33 day intervals from all feeding ports on each feeder. Feeding port samples were pooled to provide 30 approximate 100 g samples each month. Feeders were filled using two shipments of grain during the entire feeding period each year. Because new grain was added to grain already present in feeders, pre-residence grain samples could not be evaluated. Instead, two 200 g samples were obtained by intermittently collecting grain from the inflow stream of each shipment. The stream samples were pooled to provide one sample for each grain shipment. The first shipment of grain was used to fill feeders initially and the second was stored in overhead feed bins until needed. Moisture content of grain stored in overhead bins remained below 10% at all times. The study was designed as a repeated measures split-plot with individual feeders as the blocked variable. Month was in the top of the split plot and year in the bottom. Repeated measures was used to account for the violation of independent samples because samples were taken

from the same feeders over time (von Ende, 1993).

After grain samples were collected, they were stored on ice (<8 hr) until they were frozen (−20 C). Samples were stored at or near a frozen state until they could be freeze-dried to prevent fungal growth. Frozen samples were ground to pass a #20 mesh using a Wiley-Mills 3375-E10, model #4 grinder (Arthur H. Thomas Co., Philadelphia, Pennsylvania, USA). The grinder was thoroughly cleaned between samples to avoid cross-contamination of samples. After grinding, samples were homogenized and returned to a freezer. A 10 g sample of each homogenate was placed in a petri dish and dried to a constant weight using an FTS Systems Dura-Top Bulk Drier, model #TD-4A-0 (FTS Systems, Inc., Stone Ridge, New York, USA). Samples were then stored in a dessicator until analyzed for AF content.

Aflatoxin concentrations of grain samples were determined using Neogen Veratox® kits (Neogen Corporation, Lansing, Michigan, USA). Neogen Corporation's recommendations for optimal retrieval of AF were followed. Briefly, a 10 g sample of each homogenate was mixed with 50 ml of an extraction solution (70% methanol and 30% water) and shaken vigorously for three minutes. This mixture was then filtered through #2 filter paper into a collection bottle. One hundred μl each of a 0, 5, 15, and 50 ppb AF control and 100 μl of each sample extraction was pipetted into separate mixing wells containing 100 μl of conjugate. Each well was then mixed to assure homogenization within wells. One hundred μl from each well was transferred to antibody-coated wells and incubated for 2 min. Wells were then washed with a steady stream of deionized water and tapped out on a paper towel until dry. One hundred μl of reaction reagent was added and allowed to incubate 3 min. Next, a stopping solution was added to the wells and thoroughly mixed. Aflatoxin concentration was quantified using the Bio-Tek EL-301 microwell reader (Neogen Corporation, Lansing, Michigan, USA). Minimum, maximum, and average monthly ambient temperature, relative humidity, and precipitation values were obtained from the Oklahoma Mesonet station (Cheyenne, Oklahoma) approximately 32 km from the study site.

Statistical analysis

When the data were analyzed, some data were missing because feeders occasionally had no feed in them at the sampling date. Therefore, least squared means were used for analysis. Data were square root transformed, because of difficulty satisfying the assumptions of normality, sphericity, and no block × treatment interaction. This approach satisfied the assumptions of sphericity and no block × treatment interaction. Although normality was not wholly met, the Shapiro-Wilks value (ranges from 0 to 1, 1 being perfectly normal) for error A was 0.95 and for error B was 0.96. Because the distribution of data was relatively symmetric around the mean and the F-test is relatively robust to violations of normality (Pearson, 1931), normality was assumed to be satisfied at this point and mean separation was completed. One sample *t*-tests (Kirk, 1990) were conducted between the pre-sample AF test of years 1 and 2 and the corresponding average monthly AF concentration in November of years 1 and 2 to determine if AF increased in supplemental feeders with feed that had not been in storage. Multiple regression was used to determine if monthly average AF production in feeders could be predicted from minimum, maximum, and average monthly ambient temperature, relative humidity, and precipitation values (Ott, 1988).

RESULTS

AF was not detected in the grain shipments used to fill feeders. Mean monthly AF concentration of samples from feeders ranged from 0.57 to 15.47 ppb (Fig. 1). The highest concentration detected in an individual sample was 157 ppb (November 1997). Mean AF concentration of samples differed ($P < 0.05$) among months, however, the general trend of AF production in year 1 was not similar to the trend in year 2 (Fig. 1). One-sample *t*-tests (Kirk, 1990) between the pre-sample of year 1 (0 ppb) and year 2 (0 ppb) and the average monthly AF concentration in November of year 1 (5.38 ppb) and year 2 (7.74 ppb) indicated that there was an increase ($P < 0.05$) from pre-sample to one month after filling the feeders each year. Additionally, AF production in feeders was compared within years 1 and 2 and within months, across years 1 and 2 (Fig. 1). Changes were detected through the year and between months; however, the general trend of AF production in supplemental feeders in year 1 was not similar to the trend in year 2. Average relative humidity was the only weather variable that contributed (*P*

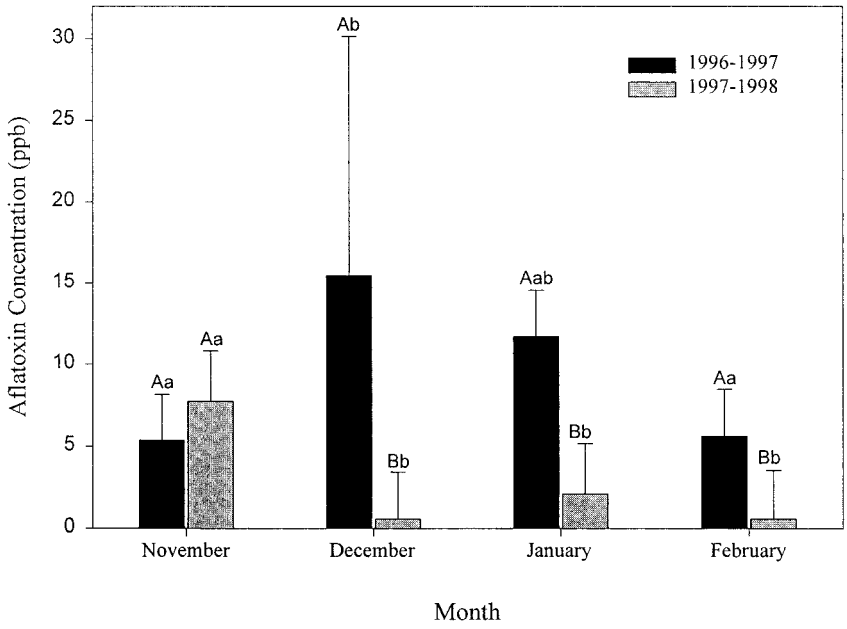

FIGURE 1. Mean aflatoxin production in supplemental feeders from November through February of 1996–97 and 1997–98. Different lower case letters indicate differences among months within year 1 and year 2, and different capital letters indicate monthly differences between year 1 and 2 ($P < 0.05$).

< 0.05) to the multiple regression prediction equation ($Y = 45.4 - 0.61x$; $R^2 = 0.905$).

DISCUSSION

Data from this study support the conclusion that AF can be produced in supplemental feeders. This conclusion is not an artifact of AF production in storage bins before placement in feeders. Pre-residence grain samples could not be evaluated throughout the study, but AF concentration of grain increased from pre-sample to one month after filling the supplemental feeders during both years of the study. Further, AF production in supplemental feeders was highly variable among months with no real temporal pattern between years. Instead, AF production was related to the highly variable relative humidity of the study area which influences moisture content of grain. Relative humidity would not have the same impact on grain in storage bins, because fans were used to keep moisture levels in bins low.

Some individual samples of grain in feeders from this study contained concentrations of AF greater than 100 ppb; the level allowed by the U. S. Food and Drug Administration (Washington, D. C., USA) for poultry feed (http://vm.cfsan.fda.gov/~lrd/fdaact.html#afla) and by the Texas State Feed and Fertilizer Control Service (Austin, Texas, USA) for supplemental wildlife feed (http://www.tpwd.state.tx.us/news/news/980907d.htm). However, mean AF levels of grain in feeders are less than those found to cause mortality or clinical signs symptomatic of aflatoxicosis in controlled laboratory experiments (Ruff et al., 1992). These levels cannot be readily interpreted as having no adverse effects on northern bobwhite, because there has been little examination of the effects of low levels of AF exposure to northern bobwhite in the field environment. However, low levels of AF (200 ppb) have been found to affect the embryonic development of broiler chicks and their ability to fight infection later in life (Qureshi et al.,

1998). Because chickens are more resistant to AF than northern bobwhite (Arafa et al., 1981), it may be expected that the levels found in feeders during this study could deleteriously affect northern bobwhite. Other sympatric species could also be impacted by AF in supplemental feeders, because it is possible for a variety of wildlife to use these feeders. Further, AF cause reductions in metabolic efficiency, with severity related to dose (Carnaghan and Allcroft, 1962; Donaldson et al., 1972). Most wild species rely on highly efficient metabolic systems to survive harsh environments (Robbins, 1983). Low levels of AF that do not cause morbidity in controlled laboratory settings may reduce the probability of survival of individuals that rely on metabolic efficiency to cope with extreme environments.

Supplemental feeders provide suitable environmental conditions for AF production. Data from this study suggest it may be possible to predict AF production in supplemental feeders using relative humidity. It is not clear, however, how our conclusions may apply to other locations with different climatic conditions. It is possible corn contaminated with AF at the beginning could produce higher, lethal levels of AF while in residence in a feeder. If a land manager chooses to incorporate supplemental feeders into a management plan, the feeders should be filled with aflatoxin-free grain and carefully maintained by promptly removing spoiled feed and periodically disinfecting the feeders.

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