BioOne COMPLETE

EVALUATION OF LOW-LEVEL AFLATOXIN IN THE DIET OF WHITE-TAILED DEER

Authors: Quist, C. F., Howerth, E. W., Fischer, J. R., Wyatt, R. D., Miller, D. M., et al.

Source: Journal of Wildlife Diseases, 33(1) : 112-121

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-33.1.112

BioOne Complete (complete.BioOne.org) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/terms-of-use.

Usage of BioOne Complete content is strictly limited to personal, educational, and non - commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

Downloaded From: https://bioone.org/journals/Journal-of-Wildlife-Diseases on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

Journal of Wildlife Diseases, 33(1), 1997, pp. 112–121
© Wildlife Disease Association 1997

EVALUATION OF LOW-LEVEL AFLATOXIN IN THE DIET OF WHITE-TAILED DEER

C. F. Quist,[1] E. W. Howerth,[2] J. R. Fischer,[1] R. D. Wyatt,[4] D. M. Miller,[3] V. F. Nettles[1]

[1] Southeastern Cooperative Wildlife Disease Study
[2] Department of Pathology, and
[3] Athens Diagnostic Laboratory; College of Veterinary Medicine; and
[4] Department of Poultry Science; University of Georgia, Athens, Georgia USA 30602

ABSTRACT: We evaluated the response of white-tailed deer (WTD) (*Odocoileus virginianus*) to dietary aflatoxin. Fourteen 4-to-5-mo-old WTD were used in this 8-wk study, conducted between November 1993 and January 1994. Seven animals received a ration containing 800 parts per billion (ppb) total aflatoxin (AF). Seven control animals received the same ration without AF. At 0, 1, 3, 6 and 8 wk, feed consumption, feed conversion, liver enzymes, bile acid levels, and immune function via lymphocyte proliferation assays and delayed type hypersensitivity reactions were determined. At the conclusion of the 8-wk feeding trial, deer were euthanized and necropsied. Clinical illness was not evident in any of the animals, but by the end of the study, AF-fed deer had reduced feed consumption and body weight as compared to control deer; the differences were not statistically significant. The AF-exposed group had a significant increase ($P = 0.03$) in serum bile acid concentration as compared to control deer. Two AF-exposed deer had gross and histologic hepatic lesions indicative of a mild degenerative hepatopathy. Residues of an aflatoxin metabolite, aflatoxin M_1, were found in the livers of all treated animals. No differences in immune function were detected between the two groups. We conclude that consumption of 800 ppb AF in the diet of young WTD over an 8-wk period can produce subclinical hepatic injury.

Key words: Aflatoxin, white-tailed deer, *Odocoileus virginianus*, liver, hepatotoxin, feeding trial.

INTRODUCTION

Aflatoxins are a family of secondary metabolites of *Aspergillus flavus* and *Aspergillus parasiticus* produced in moldy grains or seeds, such as corn (*Zea maize*), and various types of nuts under favorable environmental conditions of high moisture and warm environmental temperatures (Beasley et al., 1990). The toxic effects of aflatoxin ingestion in domestic livestock and fowl are well-known and include decreased feed consumption, decreased weight gains and feed efficiency, liver damage, immunosuppression, carcinogenesis, and in extreme cases, death (Cheeke and Shull, 1985). The U.S. Food and Drug Administration has established a maximum level of 20 ppb aflatoxin (AF) for various classes of animal feeds (Food and Drug Administration, 1989). Occasionally, grain that cannot be marketed because of excess aflatoxin has been diverted as feed for wildlife, particularly in states such as North and South Carolina (USA) where hunting white-tailed deer (*Odocoileus virginianus*) (WTD) over bait is allowed (Fischer et al., 1995). Aflatoxin levels up to 750 ppb have been found in corn taken from bait piles in these states (Fischer et al., 1995). Although adult domestic ruminants are more resistant than monogastric species to aflatoxins (Cheeke and Shull, 1985, Harvey et al., 1988), the response of non-domestic ruminants, including WTD, to dietary aflatoxin is virtually unknown.

Our objective was to determine the response of young WTD to a diet containing 800 ppb AF by monitoring feed consumption, feed conversion, and liver and immune function.

MATERIALS AND METHODS

Fourteen WTD fawns were used in this 8-wk study conducted from November 1993 to January 1994. The fawns, which ranged in age from 16 to 22 wk of age, were obtained from a local private herd (Athens, Georgia USA) and divided into two groups with approximately equivalent total body weight in each group. Deer were housed in groups of two or three animals per pen.

Half of the fawns were fed a complete deer

Downloaded From: https://bioone.org/journals/Journal-of-Wildlife-Diseases on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

ration (Purina Deer and Game Checkers; St. Louis, Missouri, USA) containing 800 ppb total AF; the remaining fawns were fed the same ration without the added AF. Feed and water were available ad libitum.

The aflatoxin used in this study was prepared by growing *Aspergillus parasiticus* NRRL 2999 on moistened polished rice according to the method of West et al. (1973). The resulting moldy rice was dried and ground to a fine powder and analyzed for aflatoxins by high performance liquid chromatography (HPLC) according to the method of Colley and Neal (1979). The rice powder had an aflatoxin content of 1.04 mg aflatoxin/g of rice powder. The ratio of the individual aflatoxins were as follows: B_1 = 74.1%, G_1 = 21.6%, B_2 = 4.0%, and G_2 = 0.3%. A weighed amount of the rice powder was added to the basal feed ration to attain the desired concentration of the dietary aflatoxin. Immediately prior to the addition of the aflatoxin rice powder, 1% food grade corn oil was added to the basal ration to minimize the dustiness of the feed. The additions were made while continually mixing the basal feed ration to insure homogeneity of the final ration. The same concentration of corn oil also was added to the control diet.

The concentration of AF in the final ration was confirmed on each batch of ration prepared. This analysis was performed with the Aflatest P affinity column procedure (Vicam, Watertown, Massachusetts, USA). The initial commercial deer diet was changed after the first 3 wk of the study in favor of a second product (F-R-M Deer Pellets; Flint River Mills, Bainbridge, Georgia) when it was determined the initial ration contained bentonite, an additive routinely used in feed manufacturing as a flow agent. This flow agent reduces the bioavailability of the added AF (R. Wyatt, pers. comm.). The substituted ration contained no flow agents. The binding of the aflatoxin by the bentonite in the initial ration resulted in a 20% reduction in target concentration of available AF. After the ration was switched, all batches of feed were analyzed and were within 5% of the desired concentration of aflatoxin.

Feed consumed by each pen of animals was measured daily. Animals were reweighed at 1, 3, 6, and 8 wk to allow determination of feed conversion on a per-pen basis. Animals were bled via jugular vein puncture at 0, 1, 3, 6, and 8 wk to obtain serum for serum chemistry profiles that included sorbitol dehydrogenase (SDH), gamma glutamyltransferase (GGT), aspartate aminotransferase (AST), total protein, creatine kinase, albumin, bilirubin, alkaline phosphatase, and bile acids. Serum analyses were performed on an Abbott Spectrum Series II analyzer (Abbott Laboratories, Dallas, Texas, USA). Bile acid levels were determined using a commercially available kit (Enzable^R, Nycomed Pharma AS, Oslo, Norway).

To assess humoral immune function, serologic response to tetanus toxoid vaccine was evaluated. All WTD were vaccinated intramuscularly with 1 ml tetanus toxoid vaccine (Tetmune with DuoPhase, SmithKline Beecham Corporation, West Chester, Pennsylvania, USA) 6 wk into the study. Serologic conversion was evaluated by comparison of pre-vaccination tetanus toxoid antibody titers to antibody titers detected at 8 wk. Antibody titers were measured using both a passive hemagglutination inhibition assay and an enzyme-linked immunosorbent assay (ELISA). The passive hemagglutination assay was performed according to the techniques of Boydon (1951). In the ELISA test, 96-well culture plates were coated with 50 µl/well of a 1:40 dilution of non-alum precipitated tetanus toxoid (Burroughs-Welcome Company, Research Triangle Park, North Carolina, USA). Plates were incubated for 30 min at 37 C, then held overnight at 4 C. The plates then were blocked with 0.5% non-fat dry milk in phosphate-buffered saline (PBS) for 30 min at 37 C, and rinsed. All deer sera were heat-inactivated in a 56 C water bath for 30 min and used at dilutions beginning at 1:5. Tetanus antitoxin (Fort Dodge Laboratories, Inc., Fort Dodge, Iowa, USA) was used as a positive control at a dilution of 1:2,400. Conjugates used were rabbit anti-horse IgG-horseradish peroxidase (HRP; Sigma Chemical Company, St. Louis, Missouri) for the positive control, and rabbit anti-deer IgG-HRP (Kirkegaard and Perry Laboratories, Inc., Gaithersburg, Maryland, USA) for the deer sera. Prior to being read on an ELISA reader, conjugates were incubated for 30 min at 37 C, then treated with O-phenol diamine substrate (Eastman Kodak Company, Rochester, New York USA) and incubated for 30 min at 25 C.

Cell-mediated immunity was assessed by lymphocyte proliferation assays and delayed type hypersensitivity response tests. Lymphocyte proliferation assays were performed at 0, 1, 3, 6, and 8 wk. The assay, based on the technique of Ghalib et al. (1985), was previously adapted for WTD lymphocytes. Briefly, 9 ml of blood collected in sodium citrate was centrifuged at 250 × *G* for 10 min and the plasma was removed. The concentrated blood cells were resuspended in Dulbecco's PBS (DPBS, Sigma Chemical Company) layered over 5 ml Histopaque 1077 (Sigma Chemical Company), and centrifuged at 400 × *G* for 30 min. The separated cell layer was examined microscopically and determined to be >95% lymphocytes,

Downloaded From: https://bioone.org/journals/Journal-of-Wildlife-Diseases on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

which were washed three times with DPBS. Cell viability was routinely >95% as determined by exclusion of trypan blue dye (Flow Laboratories, Flow General Company, McLean, Virginia, USA). Mononuclear cells were placed in 96-well microtiter plates at a concentration of approximately 2×10^5 cells/well in culture media consisting of Minimal Essential Media (MEM) with 5% fetal bovine serum, 200 mM/l L-glutamine, 1×10^5 U/l penicillin, and 10 mg/l dihydrostreptomycin (Sigma Chemical Company). Previously established optimal concentrations of two mitogens, concanavalin A (Con A; Sigma Chemical Company) at 5 and 10 μg/ml and phytohemagglutinin (PHA; Sigma Chemical Company) at 5 and 10 μg/ml, were added to the wells. Each concentration as well as control wells were tested in triplicate. Plates were incubated for approximately 3.5 days in 5% CO_2 at 37 C. Cultures then were incubated for 18 hr with 0.1 microCuries/well of tritiated thymidine (Dupont NEN Research Products, Boston, Massachusetts). Cells were harvested onto glass fiber filters using a semi-automated cell harvester (Skatron 11029, Skatron Instruments, Inc., Sterling, Virginia) and suspended in biodegradable scintillation fluid (Scintiverse-BD, Fisher Scientific Company, Pittsburgh, Pennsylvania, USA). Radioactivity was counted in a liquid scintillation counter (Beckman LS 3801, Beckman Instruments, Fullerton, California, USA). Results were expressed as the average counts per minute (CPM) of mitogen-stimulated wells minus the baseline CPM of control wells.

Delayed type hypersensitivity response was measured on all deer at 0, 6, and 8 wk. Phytohemagglutinin was used as a non-specific in vivo mitogen to test the effector arm of the hypersensitivity response as described by Smith and DeShazo (1992). An area approximately 10 cm^2 in size was clipped on the necks of the deer and two approximately 2 × 2 cm squares were drawn with an indelible marker. The initial skin-fold thickness of each site was measured using Vernier calipers. The amount of intradermal mitogen had been previously titrated in normal deer to determine optimal concentration and response time. Deer were intradermally inoculated at one site with 100 μl phytohemagglutinin diluted to 1 mg/ml in DPBS; 100 μl of DPBS was injected in the adjacent location to serve as a control site. Skin-fold thickness at each site was remeasured 48 hr after injection. The response to the mitogen was determined by subtraction of initial skin thickness measurements from 48 hour post-injection measurements.

At the end of the 8-wk study, deer were weighed, euthanized with intravenous pentobarbital (2.0 ml/kg; Butler Company, Columbus, Ohio USA) and age was determined according to the method of Severinghaus (1949). Gross necropsy examinations were performed on all organ systems. Weights were obtained on liver, spleen, kidneys, and thymus. Fifty-gram samples of liver and skeletal muscle were snap frozen in liquid nitrogen and stored at −70 C, pending residue analysis. Tissues collected for histologic examination included lymph node, reticulum, rumen, abomasum, small and large intestine, lung, liver, and kidney. Sections of liver were sampled from the same location of the right and left liver lobe in all animals. Tissues were fixed in 10% buffered formalin, embedded in paraffin, sectioned at 4 μm, and stained with hematoxylin and eosin (H&E). Both liver sections were stained with H&E; sections from the left liver lobe were stained with a reticulum and a Masson's trichrome stain (Luna, 1968).

To assess histopathologic changes, these sections of the right and left liver lobes from all animals were blindly evaluated by three pathologists (EWH, JRF, DMM). A scoring key (Table 1) was developed based on previous studies with swine (Miller et al., 1981). After the pathologists initially determined that sections from the right and left liver lobe had similar histologic features, final analysis was performed on the left liver lobe section from all animals. Twenty high power fields (450×) from sections of the left liver lobe were examined and a final score was derived for each animal by summation of scores in each category as assessed by all pathologists (Table 2).

Aflatoxin B_1 and M_1 levels in samples of liver and skeletal muscle of AF-exposed WTD were measured at the National Veterinary Services Laboratory in Ames, Iowa, via thin layer chromatography using Association of Official Analytic Chemists' techniques (Helrich, 1990). The results were reported as falling within various detectable ranges.

The feed consumption and feed conversion data were analyzed using a two-factor analysis of variance, with group and time as variables. The serum chemistry data was analyzed using a three-factor analysis of variance (Zar, 1974). The factors analyzed included AF-exposure as a group, values of each individual animal, and week of sample collection. If a significant difference was found in any factor, a subsequent analysis was performed using a Tukey multiple comparison test (Zar, 1974). Scores from the pathologists were analyzed using a three-factor analysis comparing scores from each pathologist, AF-exposure of the group, and scores for each individual deer. Statistical correlations in remaining observations were determined by comparison of the AF-exposed group to the

Downloaded From: https://bioone.org/journals/Journal-of-Wildlife-Diseases on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

TABLE 1. Key used for histopathological scoring of liver lesions seen in control and aflatoxin-exposed white-tailed deer.

Karyomegaly: nuclear diameters greater than approximately 11 μm (mean hepatocyte nuclear diameter 7.1 μm)
- 0 = No lesions present
- 1 = Less than 10 cells with karyomegaly/40 high power fields (HPF)
- 2 = Greater than 10 cells with karyomegaly/40 HPF fields

Hepatocellular degeneration: swelling, increased eosinophilia, granularity and vacuolation of hepatocytes
- 0 = No lesions present
- 1 = Mild hepatocellular degeneration
- 2 = Marked hepatocellular degeneration

Binucleate cells
- 0 = <1 binucleate cells per field (average of 20 HPF)
- 1 = from 1 to 2 binucleate cells per field (average of 20 HPF)
- 2 = from 2 to 3 binucleate cells per field (average of 20 HPF)
- 3 = 3 or more binucleate cells per field (average of 20 HPF)

Bile duct proliferation
- 0 = No bile duct proliferation
- 1 = Mild bile duct proliferation
- 2 = Marked bile duct proliferation

Periportal fibrosis
- 0 = No fibrosis
- 1 = Mild periportal fibrosis
- 2 = Moderate periportal fibrosis
- 3 = Marked periportal fibrosis

Inflammation
- 0 = No significant inflammation
- 1 = Mild inflammation confined to portal regions
- 2 = Moderate inflammation confined to portal regions
- 3 = Inflammation expanding beyond portal regions

TABLE 2. Mean (± SD) histopathology score per category of lesions in livers from white-tailed deer fed 800 ppb aflatoxin. The mean score in each category was calculated by summation of the pathologists' scores for all deer in each category, then dividing by the number of deer evaluated in each group (n = 6). The total score was calculated by adding the scores from all the pathologists in all categories then dividing by the number of deer in each group (n = 6).

Lesion category	Aflatoxin-exposed deer	Control deer
Karyomegaly	3.75 ± 2.36	1.67 ± 0.52
Hepatocellular degeneration	3.33 ± 2.73	1.83 ± 1.16
Binucleate cells	2.75 ± 1.60	2.50 ± 1.05
Bile duct proliferation	1.67 ± 0.82	1.67 ± 1.21
Fibrosis	1.67 ± 0.52	2.25 ± 0.76
Inflammation	2.83 ± 1.21	3.08 ± 0.66
Total score	16.0 ± 2.57	13.0 ± 1.15

control group using the Student's t-test. Significance level used was $P < 0.05$.

RESULTS

Overt clinical illness attributable to aflatoxicosis was not observed. One AF-exposed WTD died suddenly from an acute streptococcal meningitis 10 days into the study. The death of this deer did not appear to be related to the AF exposure. Until the day of its death, this animal had not appeared clinically ill, nor had it obviously decreased its feed consumption until the day of its death.

By the end of the study, the mean (±SD) feed consumption of the AF-exposed deer (0.41 ± 0.14 kg of feed/day) was lower than that of the control WTD (0.50 ± 0.28 kg of feed/day); initial feed consumption had been virtually identical

Downloaded From: https://bioone.org/journals/Journal-of-Wildlife-Diseases on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

TABLE 3. Blood chemistry values of control and aflatoxin-exposed (AF) white-tailed deer over the 8 wk study period. Only values in which statistically significant alterations in levels at some time point within the study are shown.

	Week				
Clinical parameter	0	1	3	6	8
Aspartate amino-transferase					
AF deer:	436 ± 455[a]	177 ± 62	124 ± 32	131 ± 36	133 ± 41
Control deer:	226 ± 140	142 ± 66	182 ± 113	113 ± 28	100 ± 22
Bile acids					
AF deer:	10.6 ± 6.4	14.5 ± 10.5	16.5 ± 16.2	34.1 ± 43.8	35.5 ± 45.1
Control deer:	11.1 ± 3.8	14.0 ± 8.0	16.6 ± 7.3	12.5 ± 7.1	9.0 ± 3.3
Creatine kinase					
AF deer:	4,338 ± 6,628	252 ± 64	222 ± 127	248 ± 73	226 ± 77
Control deer:	1,434 ± 969	209 ± 84	200 ± 89	298 ± 161	224 ± 85
Total bilirubin					
AF deer:	0.23 ± 0.14	0.31 ± 0.09	0.48 ± 0.20	0.38 ± 0.26	0.30 ± 0.25
Control deer:	0.30 ± 0.18	0.24 ± 0.05	0.30 ± 0.08	0.21 ± 0.04	0.20 ± 0.05

[a] Mean ± SD.

betweens the groups (AF-exposed at 0.818 kg of feed/deer/day versus the control deer at 0.823 kg of feed/deer/day). This slight difference in mean feed consumption of the AF-exposed group corresponded to a slightly lower mean body weight of AF--exposed deer by the end of the study. Mean (±SD) deer body weight at the beginning of the study was 19.86 ± 4.84 kg for the AF-exposed group and 19.77 ± 5.10 kg for the control group. By the end of the study, the mean (±SD) body weight of the AF-exposed deer was 20.60 ± 4.14 kg compared to 21.56 ± 3.60 kg for the control deer. Feed conversion for the AF--exposed deer as a group was 0.92 kg feed/kg gain compared to 0.87 kg feed/kg of gain for the control deer group. The differences in body weights or feed consumption were not statistically significant.

Although mild increases were seen in liver-specific enzymes during the middle of the study, only bile acid levels, which reflect liver function, were persistently and statistically increased in AF-exposed deer (Table 3). Overall, the mean (±SD) bile acid concentration of AF-exposed deer (22.4 ± 3.14 μmol/l) was significantly (P = 0.03) greater than the mean of the control deer (12.6 ± 2.90 μmol/l). The differences were attributable to increased bile acid levels in four of the six surviving AF-exposed animals; two animals had little variation in bile acid levels during the study. One animal had a mean (±SD) bile acid concentration (63.6 ± 7.68 μmol/l) during the course of the study that was significantly higher ($P < 0.01$) than any other deer. At necropsy, dental eruption patterns combined with lower initial weights were evidence that the fawns with increased bile acid concentrations were among the youngest of the AF-exposed animals.

Mean (±SD) total serum bilirubin concentrations of the AF-exposed deer (0.35 ± 0.025 mg/dl) were significantly (P = 0.008) increased over the mean of control deer (0.25 ± 0.023 mg/dl). Levels began increasing by wk 1, peaked at wk 3, then decreased by wk 8 (Table 3). The degree of increase was slight and icterus was not detected clinically. Average levels of enzymes associated with hepatocellular damage (SDH, GGT, or alkaline phosphatase) also increased slightly by 3 wk into the study, then decreased. However, these elevations were not statistically significant when compared to control deer. Aspartate

Downloaded From: https://bioone.org/journals/Journal-of-Wildlife-Diseases on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

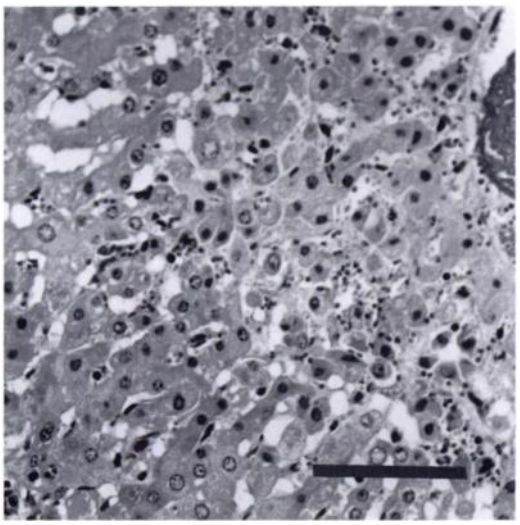


FIGURE 1. Photomicrograph of liver section from an aflatoxin-exposed whitetailed deer wherein widespread hepatocellular degeneration and necrosis resulted in partial collapse of lobular architecture. H&E. Bar = 65 μm.

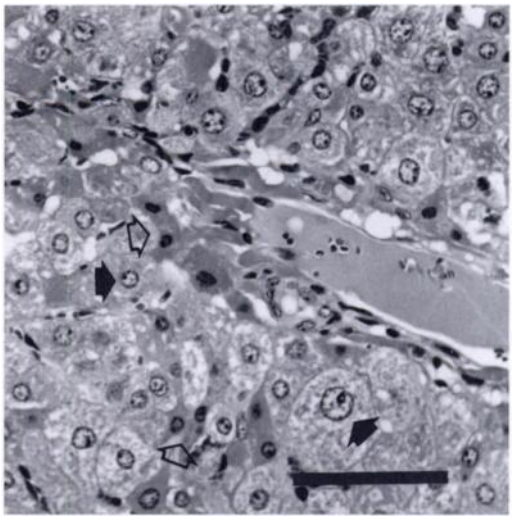


FIGURE 2. Photomicrograph of liver section from an aflatoxin-exposed whitetailed deer. Solid arrows indicate variation in nuclear size of hepatocytes. Open arrows indicate increased eosinophilia and nuclear pyknosis of hepatocytes. H&E. Bar = 50 μm.

aminotransferase (AST) activities were significantly ($P < 0.01$) increased in deer of both groups at the initial sample point (wk 0). However, AST activity in all deer decreased steadily over the course of the study; thus initial increases were probably associated with increased muscle enzyme activity secondary to capture and transfer of the animals to the research facility. The initial increases in creatine kinase levels which also are indicative of muscle damage support that assessment. The greater increases seen in both AST and CK in the AF-exposed group are primarily due to extreme increases seen in the serum from one individual, and are not indicative of any meaningful differences between the two groups at this time point.

Only two of six AF-exposed WTD had grossly detectable liver lesions; these two animals also had the highest average bile acid levels. The livers of these two deer were pale, firm, and had slightly rounded borders. No other treatment-related gross lesions were seen in any of the animals.

The mean weights of liver, kidney and spleen in AF-exposed WTD were slightly greater than those of control deer, and thymic mass was slightly less than controls (data not shown); however, the differences in organ weights between the AF-exposed WTD and control WTD were small and not statistically significant.

Based on pathologists' scores, histological lesions detected in AF-exposed WTD were primarily in two of the six categories, hepatocellular degeneration and karyomegaly (Table 2). Histologic lesions were detected in the livers of the two fawns with grossly detectable liver lesions (Figs. 1, 2). Similar degenerative liver changes were present in both fawns, but were more marked in one animal. Multifocal to confluent degeneration of hepatocytes, which was particularly evident surrounding centrilobular veins, was apparent throughout sections from the right and left hepatic lobe. Degenerate hepatocytes had increased cytoplasmic eosinophilia and occasional vacuolar degeneration. Cell nuclei varied in size with occasional megakaryocytosis (cell nuclei > 11 μm). Nuclear pyknosis was common. Stains of reticulum fibers on liver sections from these deer confirmed the loss of normal hepatocellular architecture and disarray in plates of

Downloaded From: https://bioone.org/journals/Journal-of-Wildlife-Diseases on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

degenerate hepatocytes with little increase in the supporting connective tissue network. Liver sections of remaining AF-exposed animals could not be differentiated from those of control deer. Bile duct proliferation and periportal fibrosis were not found in any WTD. Lesions were not detected in kidneys, lungs, lymph nodes, rumen, reticulum, or large or small intestinal tract.

No trends or statistically significant differences were noted between the responses of control and AF-exposed groups in humoral or cell-mediated immune function tests. Serologic conversion after the single tetanus toxoid vaccine was evident in two of seven control deer (both had titers of 1:80) and four of seven AF-exposed deer (titers ranged from 1:20 to 1:80) as measured by ELISA, which proved more sensitive than the hemagglutination inhibition assay (highest titer seen was 1:20). Had time allowed, a booster vaccine may been beneficial in evaluation of the humoral immune response. By 8 wk, the mean ± SD in vitro proliferative response of lymphocytes to both Con A and PHA remained similar between aflatoxin-exposed deer (89,227 ± 50,821 net cpm) and control deer (87,170 ± 33,847 net cpm). Similarly, the in vivo response ($\bar{X}$ ± SD) to intradermal PHA in delayed type hypersensitivity responses was not significantly different between AF-exposed deer (7.58 ± 2.4 mm) and control deer (7.78 ± 2.9). One of the deer that had increased bile acid levels had a decreased in vitro but not in vivo lymphocytic proliferation to PHA, but as one control deer had a similar response, no trends could be identified.

There were no detectable AF B_1 residues in liver and skeletal muscle from AF-exposed WTD. Low levels of AF M_1 (1 to 2 ppb), a metabolite of AF B_1, were found in the liver of five of six AF-exposed fawns. The liver of one fawn contained >2 ppb AF M_1; the small sample volume precluded reanalysis to determine the precise level of AF M_1. Residues of 0.5 to 1.0 ppb AF M_1 were found in skeletal muscle of one of six AF-exposed WTD.

DISCUSSION

Aflatoxins are more toxic to young than adult animals (Pier, 1992). Here, fawns with the most pronounced liver lesions were among the smallest of the AF-exposed group and appeared to be among the youngest deer used in the study, though the fawns used in the study probably varied in age by only a few weeks. Nonetheless, the youngest deer may be at greatest risk from the effects of AF.

In sheep and cattle, increased serum bile acid concentration is the most sensitive indicator of hepatic dysfunction. In diffuse hepatic diseases, increased bile acid concentration often precedes an increase in liver-specific enzymes, such as SDH and GGT, or bilirubin (West et al., 1987; West, 1991). Furthermore, increased bile acid concentration is the most sensitive indicator of widespread hepatic necrosis (West et al., 1987). Increased bile acids have been reported in steers experimentally fed aflatoxin for 15 wk with only early transient elevations in SDH (Richard et al., 1983). Similarly in our trial, WTD that had significant serum bile acid level increases had only mild increases in bilirubin early in the study. Richard et al. (1983) theorized that an early elevation of SDH levels seen in cattle from their study, though transient, may have indicated some early hepatocellular injury that eventually repaired and tolerated further injury.

Hepatic biotransformation enzymes of the P-450 mixed-function oxidase system can be induced by a toxin, enabling more rapid transformation of that toxin, or suppressed by a toxin, which can allow increased tolerance of certain compounds (Kelly, 1993) such as aflatoxins, in which the metabolic products also are toxic (Cheeke and Shull, 1985). The initial lower dose of the AF fed these deer may have cause some hepatic damage as evidenced by the slight elevation in bilirubin levels, but enabled biotransformation systems, re-

Downloaded From: https://bioone.org/journals/Journal-of-Wildlife-Diseases on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

sulting in hepatic recovery and relative tolerance to further damage. It is probable that only the most susceptible animals later developed sufficient functional hepatic injury to result in increase of bile acid levels.

Histopathologically, the WTD with significant serum bile acid increases had lesions compatible with early hepatocellular damage similar to those seen in other species with mild chronic aflatoxicosis (Cheeke and Shull, 1985). Histologic changes indicative of early hepatocellular degeneration include increased cellular eosinophilia, piecemeal necrosis with nuclear pyknosis, and rare vacuolization (Ruebner and Montgomery, 1982). The classic lesions associated with severe chronic aflatoxicosis, severe periportal fibrosis and biliary duct hyperplasia (Cheeke and Shull, 1985), were not seen in our study animals. Chronic lesions attributable to aflatoxicosis have been seen in deer (W. R. Davidson, pers. comm.). Severe periportal fibrosis and biliary duct hyperplasia was seen in a wild WTD that had access to a large pile of moldy corn; it was diagnosed as chronic aflatoxicosis. The season of the year and feeding habits of WTD precluded including *Crotalaria* sp., the most common pyrrolizidine alkaloid plant found in the area, as a likely cause of the hepatic lesion.

By the end of the study, feed consumption was decreased in AF-exposed WTD and there was a minimal increase in feed conversion, which corresponds to findings in cattle (Helferich et al., 1986) and pigs (Harvey et al., 1988). In ruminants, reduced feed consumption was an early event that preceded decreased rates of gain or weight loss (Cheek and Shull, 1985). Harvey et al. (1988) suggested that the reduced feed intake associated with lower doses of AF is offset by an improvement in feed utilization. The feed consumption data of the AF-exposed group was not affected by the loss of the single AF-exposed fawn that succumbed to streptococcal meningitis early in the study as that fawn died prior to any noted differences in feed consumption between the groups.

Aflatoxins selectively impair cell-mediated immunity while sparing humoral immune responses unless very high levels of aflatoxin are fed (Cheek and Shull, 1985; Pier, 1992). Thus, the lack of significant differences in serological conversion to the single vaccination of tetanus toxoid was not unexpected. The failure to detect differences in the cell-mediated immune response here is likely due to the duration of exposure, the inherent variability in lymphocyte proliferative responses from outbred animals, and what apparently is a relatively low AF dose for this species. Although use of PHA in the delayed type hypersensitivity test correlates with other measures of immune function (Mendenhall et al., 1989), the use of a specific antigen such as tuberculin PPD in this test might have proved a more sensitive indicator of immune function. Higher or more prolonged exposure of WTD to AF may be required for significant immune alterations to be detected.

Aflatoxin residues found in AF-treated WTD in this study were similar to those documented in similar studies in cattle (Richard et al., 1983; Helferich et al., 1986). Here, as in those studies, aflatoxin M_1 was the primary metabolite found, with liver containing the highest amounts. Aflatoxin B_1 is rapidly metabolized into aflatoxin M_1 and other metabolites by the cytochrome P-450 linked mixed function oxidase system of the liver (Cheek and Shull, 1985). In sequential sampling studies using AF-fed steers, there were no detectable levels of AF in liver samples collected 14 days after withdrawal from feed contaminated with 600 ppb AF (Helferich et al., 1986). It is likely that the residues found in deer also would disappear rapidly.

From these data, we propose that the dosage of 800 ppb AF for 8 wk may be the threshold level in regard to clinical significance to WTD. Based on these relatively small groups, few significant differences were detected between the groups of ex-

Downloaded From: https://bioone.org/journals/Journal-of-Wildlife-Diseases on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

posed and control deer; however, clinicopathological and pathological findings in individual deer were compatible with mild aflatoxicosis. We contend that consumption of 800 ppb AF in the diet of WTD fawns can result in subclinical hepatic injury. It is possible that adult deer (>1 yr) would be more tolerant of aflatoxins.

ACKNOWLEDGMENTS

We thank Dr. John Brown for assistance with statistical analysis, and Mrs. Tammy Nixon for assistance with the deer and data entry. The tetanus toxoid serology was performed by the Clinical Immunology Laboratory at the University of Georgia. The AF residue analysis was performed by the National Veterinary Services Laboratory in Ames, Iowa. This study was conducted through the sponsorship from the fish and wildlife agencies of Alabama, Arkansas, Florida, Georgia, Kentucky, Louisiana, Maryland, Mississippi, Missouri, North Carolina, Puerto Rico, South Carolina, Tennessee, Virginia, and West Virginia. Funds were provided by the Federal Aid to Wildlife Restoration Act (50 Stat. 917) through Grant Agreement 14-45-0009-94-906, National Biologic Survey, U.S. Department of the Interior.

LITERATURE CITED

BEASLEY, V. R., D. C. DORMAN, AND J. D. FIKES. 1990. A systems affected approach to veterinary toxicology, Vol. II. Illinois Animal Poison Information Center, Champaign, Illinois, pp. 485–492.

BOYDON, S. V. 1951. The absorption of proteins on erythrocytes treated with tannic acid and subsequent hemagglutination by anti-protein sera. Journal of Experimental Medicine 93: 107–120.

CHEEKE, P. R., AND L. R. SHULL. 1985. Natural toxicants in feeds and poisonous plants. AVI Publishing Company, Inc., Westport, Connecticut, pp. 403–470.

COLLEY, P. J., AND G. E. NEAL. 1979. The analysis of aflatoxin by high performance liquid chromatography. Annals of Biochemistry 93: 409–418.

FISCHER, J. R., A. V. JAIN, D. SHIPES, AND J. S. OSBORNE. 1995. Aflatoxin contamination of corn used as bait for deer in the southeastern United States. Journal of Wildlife Diseases 31: 570–572.

FOOD AND DRUG ADMINISTRATION. 1989. Corn shipped in interstate commerce for use in animal feeds; action levels for aflatoxins in animal feeds—revised compliance policy guide; availability; FDA's policy of blending of aflatoxin-contaminated corn from the 1988 harvest with noncontaminated corn for use in animal feeds. Federal Register 54, Vol. 100, pp. 22,622–22,624.

GHALIB, H. W., C. E. SCHORE, AND B. I. OSBURN. 1985. Immune response of sheep to bluetongue virus: In vitro induced lymphocyte blastogenesis. Veterinary Immunology and Immunopathology 10: 177–188.

HARVEY, R. B., W. E. HUFF, L. F. KUBENA, D. E. CORRIER, AND T. D. PHILLIPS. 1988. Progression of aflatoxicosis in growing barrows. American Journal of Veterinary Research 49: 482–487.

HELFERICH, W. G., W. N. GARRETT, D. P. H. HSIEH, AND R. L. BALDWIN. 1986. Feedlot performance and tissue residues of cattle consuming diets containing aflatoxins. Journal of Animal Science 62: 691–696.

HELRICH, K. 1990. Aflatoxins B_1 and M_1 in liver, confirmation method. Section 982.25. Official Methods of Analysis of the Association of Official Analytic Chemists, Volume II, 15th ed. The Association of Official Analytic Chemists, Inc. Arlington, Virginia, pp. 1202–1203.

KELLY, W. R. 1993. The liver and biliary system. *In* Pathology of domestic animals, 4th ed., K. V. F. Jubb, P. C. Kennedy, and N. Palmer (editors). Academic Press, Inc., San Diego, California, p. 383.

LUNA, L. G. 1968. Manual of histological staining methods of the Armed Forces Institute of Pathology. McGraw-Hill Book Company, New York, New York, p. 94.

MENDENHALL, C. L., C. J. GROSSMAN, G. A. ROSELLE, S. J. GHOSN, T. Y. COYT, S. THOMPSON, AND N. E. DEHNE. 1989. Phytohemagglutinin skin test responses to evaluate in vivo cellular immune function in rats (42838). Proceedings of the Society for Experimental Biology and Medicine 190: 117–120.

MILLER, D. M., B. P. STUART, AND W. A. CROWELL. 1981. Experimental aflatoxicosis in swine: Morphological and clinical pathological results. Canadian Journal of Comparative Medicine 45: 343–351.

PIER, A. C. 1992. Major biological consequences of aflatoxicosis in animal production. Journal of Animal Science 70: 3964–3967.

RICHARD, J. L., A. C. PIER, R. D. STUBBLEFIELD, O. L. SHOTWELL, R. L. LYON, AND R. C. CUTLIP. 1993. Effect of feeding corn naturally contaminated with aflatoxin on feed efficiency, on physiologic, immunologic, and pathologic changes, and on tissue residues in steers. American Journal of Veterinary Research 44: 1294–1299.

RUEBNER, B. H., AND C. K. MONTGOMERY. 1982. Pathology of the liver and biliary tract. John Wiley and Sons, New York, New York, pp. 33–59.

SEVERINGHAUS, C. W. 1949. Tooth development and wear as criteria of age in white-tailed deer. The Journal of Wildlife Management 13: 195–216.

SMITH, D. L., AND R. D. DESHAZO. 1992. Delayed hypersensitivity skin testing. *In* Manual of clinical laboratory immunology, 4th ed., N. R. Rose,

Downloaded From: https://bioone.org/journals/Journal-of-Wildlife-Diseases on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use

E. C. de Macario, J. L. Fahey, H. Friedman, G. M. Penn (editors). American Society for Microbiology, Washington, D. C., pp. 202–206.

WEST, H. J. 1991. Evaluation of total serum bile acid concentrations for the diagnosis of hepatobiliary disease in cattle. Research in Veterinary Science 51: 133–140.

———, A. BATES, AND G. E. HYNES. 1987. Changes in the concentration of bile acids in the plasma of sheep with liver damage. Research in Veterinary Science 43: 243–248.

WEST, S., R. D. WYATT, AND P. B. HAMILTON. 1973. Improved yield of aflatoxin by incremental increases of temperature. Applied Microbiology 25: 1018–1019.

ZAR, J. H. 1974. Biostatistical analysis, 2nd ed. Prentice-Hall, Inc., Englewood Cliffs, New Jersey, pp. 850.

Received for publication 1 February 1995.

Downloaded From: https://bioone.org/journals/Journal-of-Wildlife-Diseases on 28 Mar 2024
Terms of Use: https://bioone.org/terms-of-use