Bull. Environ. Contam. Toxicol. (1993) 50:750–756

Environmental Contamination and Toxicology

Effects of Sublethal Concentrations of Aflatoxins on the Reproductive Performance of Mink

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Aflatoxins constitute a group of structurally similar mycotoxins produced under favorable environmental conditions by certain strains of closely related fungi (primarily Aspergillus flavus and *A. parasiticus*) that grow naturally on plants in the field or on stored feedstuffs (Betina 1989). Aflatoxins are of concern because they are among the most toxic of the known mycotoxins and have been implicated in the deaths of humans and animals from consumption of moldy food (Betina 1989). Although sensitivity to aflatoxins varies from species to species (Betina 1989), studies conducted in our laboratory (Bonna *et al.* 1991), and elsewhere (Koppang and Helgebostad 1972; Chou *et al.* 1976a,b,c) have shown that mink are extremely sensitive to aflatoxins. Ingestion of 5 μg of aflatoxins per day for four weeks can produce toxic effects in mink (Koppang and Helgebostad 1972).

Previous studies pertaining to the effects of dietary aflatoxins on mink have focused primarily on growing or adult animals. To our knowledge, no research has been conducted to investigate the effects of aflatoxins on mink reproductive performance. This study was initiated to determine the effects of daily dietary exposure to sublethal concentrations of aflatoxins from naturally-contaminated feed ingredients on female mink reproduction and kit growth and survival.

MATERIALS AND METHODS

Thirty natural dark female mink (*Mustela vison*) were randomly allocated into three groups, each consisting of 10 animals, on February 18, 1991. The mink were housed individually in an indoor animal room in wire mesh cages (76 cm L x 61 cm W x 46 cm H) suspended above the floor. Each cage was equipped with an attached wooden nest box (38 cm L x 28 cm W x 27 cm H) bedded with aspen shavings and excelsior (wood wool). The photoperiod was regulated by a time clock to simulate natural

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light conditions. Ventilation was provided by an exhaust fan and ceiling vents. Temperature in the room approximated ambient temperature. Following a 10-day acclimation period, the mink were placed on the dietary treatments shown in Table 1.

Naturally-contaminated shelled corn, estimated to contain 3 ppm aflatoxins, was used in the formulation of the diets fed to the mink in groups 2 and 3. Clean corn was incorporated into the control diet (group 1). Prior to incorporation into the mink diets, the corn was finely ground and thoroughly mixed. Samples were collected and analyzed for aflatoxin B_1, B_2, G_1, and G_2 quantitatively by HPLC, as previously described (Bonna *et al.* 1991). The samples were also screened for zearalenone and deoxynivalenol. Based on the concentrations of aflatoxins in the corn, diets for groups 2 and 3 were formulated to yield targeted concentrations of 5 and 10 ppb total aflatoxins, respectively. Samples of the mixed diets were analyzed for aflatoxin (B_1, B_2, G_1, and G_2) content as described by Bonna *et al.* (1991).

Table 1. Experimental design of mink feeding trial.

Group	No. of mink	Dietary treatment
1	10	Basal diet[1] (control)
2	10	Basal diet with 5 ppb aflatoxins[2]
3	10	Basal diet with 10 ppb aflatoxins

[1] Conventional-type mink diet consisting of 22.2% commercial mink cereal, 22.2% poultry by-products, 17.8% ocean fish scrap, 6.7% eggs, 4.4% beef trimmings, and 26.7% water. The diets were each supplemented with 2 ml wheat germ oil/kg diet, 0.1 mg d-biotin/kg diet, and after April 18, 1991 with 7.6 ml corn oil/kg diet. As fed, the basal diet contained 14.1% protein, 8.89% fat, 3.82% ash, 1.43% crude fiber, and 59.8% moisture (analyses by Litchfield Analytical Services, Litchfield, MI 48252).

[2] Aflatoxins were incorporated into the diets fed to groups 2 and 3 via aflatoxin-contaminated corn. Appropriate quantities of a premix containing the aflatoxin-contaminated and "clean" corn ("clean" corn only for the control diet) were added to the other dietary ingredients to yield the desired aflatoxin concentrations in the feed.

Feed and drinking water were provided *ad libitum* to the mink throughout the study. Body weights of the adult females were determined at the beginning and end of the acclimation period (February 18 and 28, 1991, respectively) and at the end of the breeding season (April 4, 1991). Feed consumption was measured on February 26 and 27 and on April 2 and 3, 1991.

During the breeding season (March, 1991), the females were mated to untreated males. They were provided an opportunity to mate every fourth day until a successful mating was obtained, as verified by the presence of motile spermatozoa in vaginal aspirations taken immediately following copulation. Mated females were given the opportunity to mate a second time, either eight days after the initial mating or the next day if the initial mating occurred late in the breeding season. This procedure is in accordance with common commercial mink breeding practices. The mated females were checked daily for the birth of kits during the whelping period (April 20 through May 15, 1991). Kits were counted and weighed the day of birth and at three weeks of age.

Three kits each from different litters whelped by females in each dietary group were euthanized at birth and at three weeks of age. Their livers were weighed and samples collected for aflatoxin analysis and histopathologic examination. Three adult females from each dietary group were also euthanized and necropsied when their kits reached three weeks of age. Their organs (brain, liver, kidneys, heart, spleen and adrenal glands) were weighed and liver samples collected for histopathologic examination and aflatoxin analysis. The liver samples for aflatoxin analysis were stored frozen and those for histopathology were fixed in neutral buffered formalin and prepared for microscopic examination according to routine histologic procedures. At approximately three weeks of lactation, samples of milk were collected (Jones *et al.* 1980) from five females in each group for analysis of aflatoxin concentration. The milk and liver samples from the kits and from a dam in group 3 (10 ppb aflatoxins) and liver samples from an adult female in groups 1 and 2 were analyzed for aflatoxin M_1 (Neogen Corp., Lansing, MI) to ascertain if this primary metabolite present in milk and animal tissues was present in significant concentrations to warrant analysis of the remaining samples.

The body weight and feed consumption data were subjected to analysis of variance and significant differences ($p < 0.05$) between treatment means and the control mean were determined by Dunnett's test.

RESULTS AND DISCUSSION

Analyses of samples of the aflatoxin-contaminated corn indicated that it contained 1.90 to 2.67 ppm aflatoxin B_1 and 0.27 to 0.41 ppm B_2. No aflatoxin G_1 or G_2 (detection limit 0.004 ppm), zearalenone (detection limit 0.2 ppm), or deoxynivalenol (detection limit 0.4 ppm) was found in the corn samples. Analysis of the diets fed to the mink in groups 1 (control), 2 (5 ppb), and 3 (10 ppb) yielded aflatoxin concentrations (B_1 plus B_2) of 0.0, 2.7, and 9.7 ppb, respectively. No aflatoxin G_1 or G_2 was detected (detection limits 2.0 and 6.0 ppb for G_1 and G_2,

respectively) in the diets.

The toxicity of aflatoxins to animals is dependent upon the dose and duration of exposure as well as sex, age, and species. Toxicoses can range from death to more subtle effects such as poor feed conversion efficiency, growth suppression, and impaired immune function (Osweiler *et al.* 1976; CAST 1989). Young animals are generally more susceptible to aflatoxicosis than older animals (CAST 1989).

In the present study, feeding adult female mink sublethal concentrations of aflatoxins (B_1 and B_2) for 90 days had no negative effects on their feed consumption, body weights, mating success, gestation, or litter size. However, body weights of the kits were significantly decreased in group 3 at birth and in groups 2 and 3 at three weeks of age when compared to the controls (Table 2). Kit mortality was also greatest in the kits whelped and nursed by females fed 10 ppb aflatoxin (group 3) and reached 33% by three weeks of age. Mink kits begin to consume solid feed around 21 to 24 days of age. Thus, kit growth up to three weeks of age can provide an indication of the lactational performance of the dams. According to Joergensen (1985), the average kit mortality from birth through weaning (approximately 42 days) for commercial mink farms is 5-10%.

Aflatoxins can be transferred to feti *in utero* and to newborns via the milk (CAST 1989). The results presented in Table 2 suggest that both *in utero* and lactational exposure to the aflatoxins may have contributed to the reduced kit body weights. However, in a study conducted by Chou *et al.* (1976b), oral administration of 50 μg aflatoxin B_1 (two doses, two weeks apart) to lactating female mink had no detectable effects on the nursing kit body weights nor were aflatoxins B_1 or M_1 detected in milk samples collected 24 hours post-dosing.

Analyses of the milk samples from the control and aflatoxin-exposed animals in the present study showed relatively low concentrations of aflatoxin M_1 (a hydroxy derivative of aflatoxins B_1 and B_2 and a primary metabolite found in milk and animal tissues). Only one of the five samples of milk collected from dams fed 10 ppb aflatoxin (group 3) was above (0.29 ppb wet weight) the detection limits (0.25 ppb) for M_1. These values probably reflect the low excretion rate in milk of the total aflatoxin intake by mammals. Mertens (1979) reported that only 0.91% of the total aflatoxin intake of dairy cows was excreted in the milk. Stoloff (1979) indicated that the ratio of aflatoxin B_1 in feed to B_1 or M_1 in milk was about 300:1. Aflatoxins were not found in cows' milk after aflatoxin-treated animals received clean feed for four days (CAST 1989).

Although aflatoxins accumulate mainly in the liver and kidneys of

Table 2. Reproductive performance of female mink fed 0, 5 or 10 ppb aflatoxins and body weights and survival of their kits.

Dietary group	No. females whelping/No. females mated	Mean gestation[1] (days)	No. kits whelped		Kit body weight (g)[2]		Kit mortality birth to 3 wks[3] (%)
			Alive	Dead	At birth	At 3 wks	
1 (control)	10/10	46.2	52	5	9.5 ± 0.25	122 ± 2.5	23
2 (5 ppb aflatoxin)	10/10	45.1	51	5	9.5 ± 0.23	100 ± 2.1**	6
3 (10 ppb aflatoxin)	8/10[4]	43.3	53	4	8.5 ± 0.22**	109 ± 3.7*	33

[1] Based on date of final mating.
[2] Data expressed as mean ± standard error.
[3] Excluding the three kits from each group killed at birth for aflatoxin residue analysis and histopathology.
[4] One mated female died prior to whelping of causes unrelated to aflatoxicosis.
* Significantly different ($p < 0.05$) from control.
** Significantly different ($p < 0.01$) from control.

animals, all residues are readily cleared from the body upon termination of aflatoxin exposure (Osweiler *et al.* 1976). Chou and Marth (1976) reported only 6.6% of ^{14}C-labeled aflatoxin B_1 in female mink livers 24 hours after dosing. The concentrations of aflatoxin M_1 in the livers of the adult female mink fed 0, 5 or 10 ppb aflatoxins B_1 plus B_2 were 0.42, 0.46 and 0.30 ppb, respectively. We have no explanation for the greater concentrations of aflatoxin M_1 in the livers of the adult mink in groups 1 and 2 than in group 3. The aflatoxin M_1 concentrations in the livers from three-week-old kits from group 3 were all below the detection limits of 0.25 ppb.

The organ weights of the adult mink necropsied during the trial and liver weights of kits at birth and three weeks of age showed some trends related to aflatoxin exposure but due to the small sample size, they were not analyzed statistically. Previous studies conducted in our laboratory with mink have shown significant increases in liver and adrenal gland weights and decreases in heart weight with dietary exposure to 34 or 102 ppb aflatoxins (Bonna *et al.* 1991).

Histopathologic examination of the livers from the kits and adult females revealed no significant histological differences between the groups that could be attributed to exposure to dietary aflatoxins. However, a previous study conducted in our laboratory in which mink were fed 34 or 102 ppb aflatoxins for up to 77 days showed histopatho-logic changes in the livers of the aflatoxin-treated mink. These changes included mild to severe micro- and macro-vesiculation of hepatocytes with hepatocyte degeneration and necrosis. The lesions suggested that the early changes were periportal with subsequent progression toward panlobular or panzonal involvement (Bonna *et al.* 1991).

The results of this study suggest that low level dietary exposure to aflatoxins B_1 and B_2 does not exert a direct effect on the reproductive performance of female mink but can impair early kit growth and survival. These adverse effects on the kits may be mediated through immune function or other physiological systems.

Acknowledgments. The authors thank Dr. H.M. Stahr, Iowa State University, Ames, IA for supplying the aflatoxin-contaminated corn; Dr. W.E. Braselton, Jr. and Susan M. Stahl, Michigan State University Animal Health Diagnostic Laboratory, for analysis of mycotoxins in the feed; Mrs. Chris Bush, Mr. Angelo Napolitano, and Mr. Phil Summer for their technical assistance; and Ms. Carol Daniel for typing the manuscript. This study was supported in part by the Mink Farmers Research Foundation, Thiensville, WI and the Michigan Agricultural Experiment Station, E. Lansing, MI.

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Received July 13, 1992; accepted December 2, 1992.