Reproductive Toxicity of Ergot-Contaminated Wheat to Mink

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Introduction

Mycotoxins are toxic chemicals (metabolites) produced by fungi that can grow on plants in the field and/or on feedstuffs during storage. Over 300 mycotoxins have been identified (Betina, 1989). When consumed by animals, or humans, these toxins can be lethal or can result in severe economic losses through reduced animal productivity, increased incidence of diseases due to immunosuppression, organ damage, and/or impaired reproduction. Studies have shown that dietary exposure to several mycotoxins, including deoxynivalenal (DON), zearalenone (ZEN), T-2 toxin, aflatoxins, fumonisins, and moniliformin, can be harmful to mink (Aulerich and Bursian, 1996, Morgan *et al.*, 1999).

Ergot was one of the earliest recognized mycotoxins. It has been responsible for the deaths of thousands of humans and animals throughout history (Kilgore and Li, 1980; Betina, 1989), including mink (Cameron *et al.*, 1989). Ergot is the common term given to sclerotia (compact, dark-colored bodies, 2-20 mm long) produced by fungal species of the genus *Claviceps* that infect grasses (ryegrass, wheat grass, blue grass, and fescue) and cereal grains (wheat, rice, corn, sorghum, rye, barley, oats, and millet) (WHO, 1990; McQueen, 1993). The fungus infects the florets of grasses and grains replacing the florets with sclerotia. The sclerotia contain numerous biologically active alkaloids that are responsible for the toxicity of the mycotoxin (WHO, 1990).

Ergot alkaloids are derivatives of lysergic acid and more than 40 alkaloids have been isolated from *Claviceps* sclerotia (WHO, 1990). The different alkaloids vary in their biological activity and in the toxic effects they produce. Collectively, ergot alkaloids are called ergolines. The composition of ergolines, as well as the total ergoline content within sclerotia, can vary from plant to plant within a given field. Thus, there may be considerable variation in the toxic effects observed within an animal species exposed to feedstuffs infected with *Claviceps* sclerotia. Chronic ergotism in various animal species has resulted in swollen joints, lameness, reduced feed consumption, reduced body weights, decreased milk production, gangrene, and direct or indirect reproductive effects (McQueen, 1993).

Since a portion of the diet of ranch mink is cereal, there is concern that mink could consume significant quantities of ergot alkaloids. To our knowledge, the toxicity of ergot alkaloids to mink has not been investigated. The objective of this study was to determine and characterize the reproductive effects of dietary exposure to ergot alkaloids in mink.

Materials and Methods

Forty-eight pastel female mink were randomly assigned to 4 groups of 12 mink each. They were housed in suspended wire-mesh cages in an indoor animal room at the Michigan State University (MSU) Experimental Fur Farm beginning on February 15, 1999. The mink were housed indoors because MSU guidelines concerning the use of this mycotoxin required that the study be conducted in an approved containment facility. A wooden nest box bedded with aspen shavings and excelsior (wood wool) was attached to the outside of each cage. The room was minimally heated to keep the feed and drinking water from freezing and the lights were controlled by a timer to mimic the natural photoperiod.

The mink were acclimated to the cages and fed a basal diet for 7 days before starting the experiment. After the acclimation period, the mink were fed 1 of 4 diets containing various proportions of clean and ergot-contaminated wheat that provided 0 (control), 3, 6, or 12 ppm (mg/kg), wet weight, of ergot alkaloids. The specific ergot alkaloids were ergosine (6.8%), ergotamine (12.9%), ergocornine (14.7%), ergocryptine (16.0%), and ergocristine (49.6%). Feed and drinking water were provided *ad libitum*.

Mink feed consumption was determined daily during the first week of the trial. Body weights of the mink were recorded on days 1, 7, and 22 of the trial, at whelping, and at the end of the trial (133 days of exposure). Blood samples were collected from the jugular vein of anesthetized (ketamine HCl) mink from the control and the 12 ppm ergot alkaloid groups at the end of the trial for determination of hemoglobin concentrations, hematocrit values, and plasma chemistry parameters by the MSU Veterinary Clinical Pathology Laboratory. Blood samples, taken from all the adult females, at the end of the trial, were assessed for plasma prolactin concentration at the Diagnostic Laboratory at Cornell University, Ithaca, NY.

Beginning on March 1, 1999, the females were mated with untreated males. Matings, were verified by examination of vaginal aspirations taken immediately after mating for the presence of normal-appearing motile sperm. Mated females were given an opportunity for a second mating either the day after the initial mating or 8 days after the

Table 1. The effect of ergot alkaloids on adult female mink body weights, plasma prolactin concentrations, reproductive parameters, and kit body weights.

Parameters	Dietary concentration of ergot alkaloids (ppm)*			
	0	3	6	12
Adult female body weight change (g)	-167.3 ± 44.1 (n = 12)	-84.6 ± 44.1 (n = 12)	-176.0 ± 44.1 (n = 12)	-111.5 ± 46.1 (n= 11)
No. females bred/total no. females	11/12	12/12	12/12	11/11
No. females whelping/no. females bred	9/11	9/12	4/12	1/11
Gestation (days)	47.2 ± 2.82[a] (n = 9)	52.0 ± 2.82[a] (n = 9)	60.7 ± 4.3[b] (n = 4)	62 (n = 1)
Adult female plasma prolactin concentration 5/20/99 (ng/ml)	51.1 ± 4.2[a] (n = 12)	18.3 ± 5.1[b] (n = 8)	12.2 ± 4.2[b] (n = 12)	11.8 ± 4.6[b] (n = 10)
No. five kits/no. dead kits	33/11	12/24	1/15	0/3
Kit birth weight (g)	11.1 ± 0.4[a] (n = 33)	8.8 ± 0.7[b] (n = 12)	9.0 (n = 1)	-
Kit body weight at 3 wks (g)	113.0 ± 3.8[a] (n = 28)	65.0 ± 6.3[b] (n = 10)	-	-

*Body weights, gestation, and prolactin concentrations expressed as mean standard error. Means with different superscripts are significantly different from one another at $p < 0.05$.

first mating. Nest boxes were checked daily for kits during the whelping period (mid-April to the end of May). The kits were counted and their body weights were recorded at birth and at 3 weeks of age.

All the females were euthanized (carbon dioxide) and necropsied when the youngest kits reached 3 weeks of age. The liver, brain, heart, kidneys, spleen, and adrenal glands were removed, fixed in 10% formalin, and submitted to the MSU Animal Health Diagnostic Laboratory for histologic evaluation.

Where appropriate, the data were analyzed using the Proc Mixed Statistical Program (MSU Statistical Consulting Center). Statements of significant differences between treatment means are based on $p < 0.05$.

The use and care of the animals in this study were approved by the MSU All University Committee on Animal Use and Care. All toxic substances and wastes were handled and disposed of in accordance with procedures specified by the MSU Office of Radiation, Chemical, and Biological Safety.

Results

Feed consumption by the mink fed diets that contained ergot alkaloids was depressed for the first few days of the trial, but by day 7, the treated mink were consuming normal amounts of feed (Figure 1 on page 26). Although the mink in all groups lost body weight during the trial (Table 1), there were no statistically significant differences in body weights of the adult mink between the groups. Female mink typically lose body weight prior to and during the reproductive period (Korhonen, 1990; Hansen, 1997).

All the females fed the 3, 6, or 12 ppm ergot alkaloid diets mated successfully, while 11 of 12 females in the control (0 ppm ergot alkaloids) group mated (Table 1). Consumption of ergot alkaloids at concentrations of 6 and 12 ppm had a marked effect on the number of females that whelped. Nine of 11 mated females whelped in the 0 ppm ergot alkaloid group, 9 of 12 females whelped in the 3 ppm group, 4 of 12 whelped in the 6 ppm group, and only 1 of 11 mated females whelped in the group fed 12 ppm ergot alkaloids. Gestation (number of days from the date of the last confirmed mating to whelping) was significantly increased in the 6 ppm ergot alkaloid group compared to the control (61 vs. 47 days). The female in the 12 ppm group that whelped 3 stillborn kits had a gestation of 62 days (Table 1). Mink that had not whelped by the time the surviving kits were 3 weeks old were assumed to be barren and were euthanized and necropsied. However, in the 3 ppm ergot alkaloid group, a

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euthanized female had 6 live fetuses *in utero* on day 69 of gestation, and another female had 4 live fetuses 77 days after its last mating. A mink in the 6 ppm ergot alkaloid group had 5 five fetuses when necropsied 70 days after mating.

Consumption of ergot alkaloids caused a dose-dependent decrease in the total number of kits whelped, as well as in the number of live kits whelped. Females fed the control diet whelped 33 five kits and 11 dead kits compared to 12 live and 24 dead in the 3 ppm group, 1 live and 15 dead in the 6 ppm group, and 0 live and 3 dead kits in the 12 ppm group (Table 1). Nine deformed stillborn kits were whelped by females fed the ergot alkaloid-treated diets. The predominant deformity (in 6 of the 9 kits) was hydrocephaly. No deformaties were observed in the control kits. There were 28 kits alive in the control (0 ppm ergot alkaloid) group at 3 weeks post-whelping compared to 10 kits and 0 kits in the 3 and 6 ppm ergot alkaloid groups, respectively (Table 1).

The birth weight of control kits was significantly greater than the birth weights of kits whelped by females fed 3 ppm ergot alkaloids. At 3 weeks of age, the control kits weighed 42% more than the kits in the 3 ppm ergot alkaloid group (Table 1).

Post-whelping plasma prolactin concentrations were significantly decreased in the ergot-alkaloid-treated groups compared to the control group (Table 1). Consumption of ergot alkaloids had no significant effect on hemoglobin concentration or hematocrit. The plasma parameters in the control and 12 ppm ergot-alkaloid groups that were statistically different are presented in Table 2. Alanine aminotransferase and amylase activities were significantly decreased in the 12 ppm ergot-alkaloid group compared to the control group, while creatine kinase activity was significantly greater in the 12 ppm ergot alkaloid group than in the control group. Plasma glucose, cholesterol, chloride, and iron concentrations, and the sodium/potassium ratio in the 12 ppm ergot-alkaloid group were significantly lower than their respective concentrations in the control group, while phosphorus, potassium, and total carbon dioxide concentrations in the ergot-treated group were significantly greater than their respective control values (Table 2).

Consumption of ergot alkaloids had no significant effect on organ weights of the adult mink. Histologic examination of the tissues from the control and ergot-treated mink revealed no lesions or alterations attributable to ergot toxicosis.

Discussion

Although many effects have been reported for animals exposed to ergot alkaloids, including decreased feed intake, increased body temperature, decreased heart rate, increased respiration rate, increased blood pressure, laminitis, prolonged gestations, decreased reproductive rate, dystocia, stillborn offspring, agalactia, altered hormone

Table 2. Effect of ergot alkaloids on plasma chemistry parameters of adult female mink on 5/20/99.

		Dietary concentration of ergot alkaloids (ppm)*	
Parameter	**units**	**0 (control)**	**12**
Alanine, aminotransferase	IU/l	198 ± 20[a]	122 ± 20[b]
Amylase	U/l	88 ± 5[a]	73 ± 5[b]
Creatine, kinase	IU/l	399 ± 238[a]	1858 ± 248[b]
Cholesterol	mg/dl	257 ± 11[a]	196 ± 12[b]
Glucose	mg/dl	109± 7[a]	85 ± 7[b]
Chloride	mmol/l	121 ± 0.5[a]	119 ± 0.5[b]
Iron	µg/dl	217 ± 12[a]	163 ± 13[b]
Phosphorus	mg/dl	5.1 ± 0.3[a]	6.5 ± 0.3[b]
Potassium	mmol/l	4.8 ± 0.1[a]	5.5 ± 0.1[b]
Sodium/potassium ratio	ratio	33 ± 1[a]	29 ± 1[b]
Total $C0_2$	mmol/l	23 ± 1[a]	27 ± 1[b]

*Data presented as mean standard error. Sample size was 12 for the control group and 11 for the 12 ppm ergot-alkaloid group. Means with different superscripts are significantly different from one another at $p < 0.05$.

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concentrations, and high mortality rates, comparisons between studies may be misleading because the identity and concentration of the individual alkaloids is not always known and certain alkaloids may interact with one another to produce variable affects. Reduced feed intake has been reported for rats (Jackson *et al.*, 1996) and heifers (Thompson *et al.*, 1990) fed diets that contained ergot alkaloids. However, in another study by Jackson (1989), rats fed diets that contained ergonovine, ergocryptine, and ergotamine did not show reduced feed consumption. In our mink study, there was only an initial transient dose-dependent reduction in feed intake in the mink fed ergot alkaloids during the first few days of the trial, which was probably due to palatability rather than toxicity. Ergocryptine and ergotamine comprised about 29% of the total alkaloids in the mink diets.

The numerous reproductive complications observed in the ergot-treated mink in this study have also been reported in other species exposed to ergot alkaloids. Consumption of ergot alkaloids reduced pregnancy rates in cows by as much as 60% compared to controls (Browning *et al.*, 1997). Rats administered ergocornine, ergocryptine, and ergocristine (all present in our ergot-treated mink diets) experienced reduced pregnancy rates and an increased incidence of fetal resorptions (Griffith *et al.*, 1978). Carpent and Desclin (1969) and Griffith *et al.* (1978) reported increased fetal malformations in rats administered ergot alkaloids, while Kato *et al.* (1970) observed an increase in malformed stillborn monkeys from dams fed ergot alkaloids. In our mink study, 21% of the stillborn kits observed in the ergot-alkaloid-treated groups were deformed. However, the exact number of stillborn kits whelped is unknown because mink are cannibalistic and dams will frequently devour dead kits. Mares grazing on endophyte-infected fescue containing ergot alkaloids were reported to have increased gestations by as much as 27 days when compared to controls (Cross, 1997). Gestations in excess of 60 days, as observed in the mink fed the 6 and 12 ppm ergot-alkaloid diets, are uncommon for mink. The average gestation for mink is 49.0 to 51.3 days (Bowness, 1942; Hansson, 1947; Enders, 1952).

Prolactin is a hormone secreted by the anterior pituitary gland that stimulates and sustains lactation in postpartum mammals. Decreased prolactin concentrations have been reported in rats fed ergocornine and ergocrystine (Nasr and Pearson, 1975) and in heifers and cows administered ergotamine (Browning and Browning, 1997; Browning *et al.*, 1997, 1998). Certain ergot alkaloids, such as ergotamine, can initiate the release of dopamine, which in turn inhibits the release of prolactin from lactotroph cells in the pituitary gland adversely effecting lactation (Thompson *et al.*, 1990, Schultze *et al.*, 1999).

The decreased blood glucose concentrations observed in the ergot-treated mink may be due to the alkaloids' effect on carbohydrate metabolism, as reported for rats fed ergometrine (an ergot alkaloid not present in the ergot-treated mink diets) (Peters-Volleberg *et al.*, 1996). Decreased blood glucose levels (hypoglycemia) can result from normal hepatic glucose output with increased peripheral uptake, a decrease in hepatic gluconeogenesis combined with normal peripheral utilization of glucose,

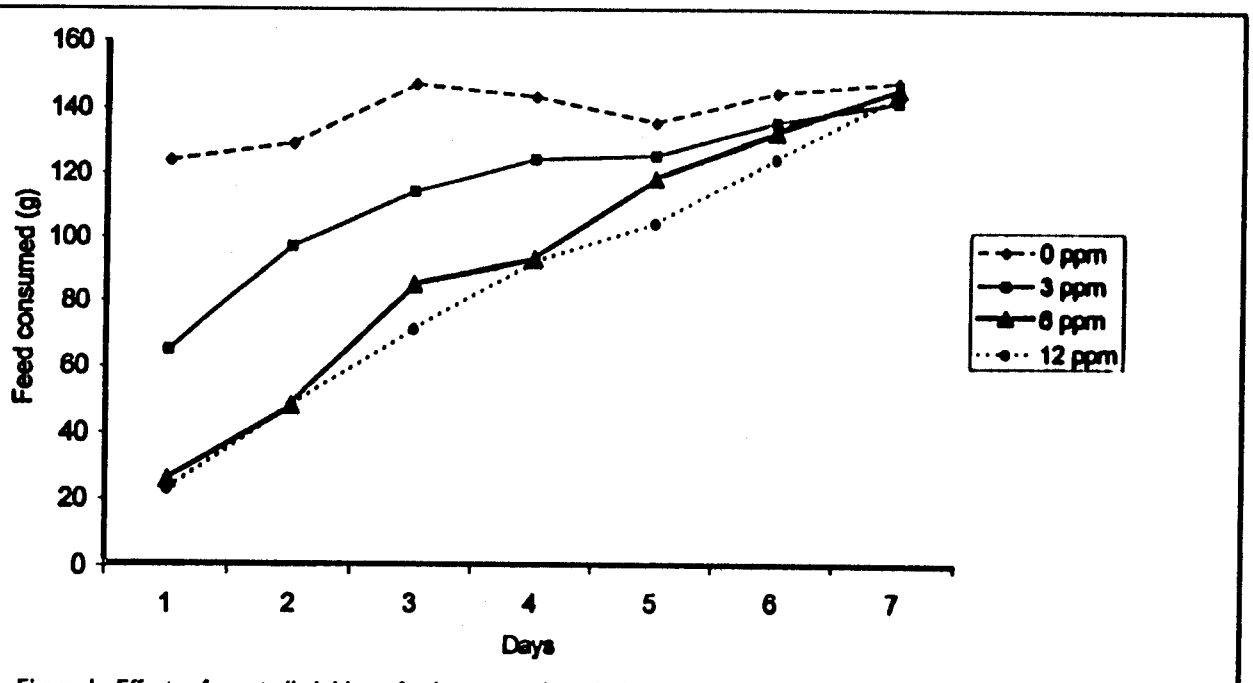

Figure 1. Effects of ergot alkaloids on feed consumption of adult female mink.

or a combination of the two mechanisms (Coles, 1986). However, hepatogenic disorders, excessive exertion, weakness, adrenal cortical insufficiency, hormonal alterations, and general hepatic dysfunction, as a consequence of circulatory deficiency and cirrhosis, can also decrease blood glucose concentrations (Coles, 1986).

The reason(s) for the significant decrease in alanine aminotransferase activity in the ergot-alkaloid-treated mink is (are) unknown, but may be associated with development of inhibitors of enzymic action, decreased enzyme production due to decreased cell proliferation, increased clearance of enzymes via the liver or kidneys, and/or alterations in conditions that may effect enzymic actions, such as pH or tonicity (Schultze *et al.*, 1999). The other statistically significant differences in plasma chemical parameters between treatments are also difficult to interpret and may not be biologically significant or may reflect the combined response of several organ systems.

Implications for Mink Farmers

Ergotism is seldom a problem today in animals fed manufactured feeds because of the improved grain handling procedures and sanitation employed by millers and feed manufacturers. However, mink feed manufacturers, especially farmers who grow and process their own cereal grains for use in mink diets, should be aware, as this study showed, that consumption of very low concentrations of ergot alkaloids by mink can result in serious negative reproductive affects.

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The 2001 International Mink Show will be held January 12-14, at the Four Points Sheraton in Milwaukee, Wisconsin.