THESIS

LIBRARY
Michigan State
University

This is to certify that the

thesis entitled

REPRODUCTIVE TOXICITY OF ERGOT-CONTAMINATED OATS IN MINK

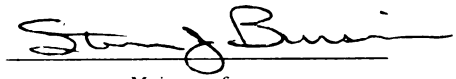
presented by

CHANDA SHARMA

has been accepted towards fulfillment
of the requirements for

M.S. degree in Animal Science

Specialization in Environmental Toxicology



Major professor

Date 12/4/01

O-7639

MSU is an Affirmative Action/Equal Opportunity Institution

PLACE IN RETURN BOX to remove this checkout from your record.
TO AVOID FINES return on or before date due.
MAY BE RECALLED with earlier due date if requested.

DATE DUE	DATE DUE	DATE DUE

6/01 c:/CIRC/DateDue.p65-p.15

REPRODUCTIVE TOXICITY OF ERGOT-CONTAMINATED OATS IN MINK

By

Chanda Sharma

A THESIS

Submitted to
Michigan State University
In partial fulfillment of the requirements
For the degree of

MASTER OF SCIENCE

Department of Animal Science

2001

ABSTRACT

Reproductive Toxicity of Ergot-Contaminated Oats in Mink

By

Chanda Sharma

Ergot alkaloids are synthesized by fungi of the Claviceps family that infect rye as well as other cereals and grains. Since a portion of the ranch mink diet is cereal, mink are at a risk of being exposed to ergot alkaloids. This study was performed to determine the reproductive toxicity of ergot alkaloids derived from ergot-contaminated oats in mink. Four groups of 12 female mink each were fed diets containing 0, 3, 6 or 12 ppm ergot alkaloids from 2 weeks prior to the breeding season until the offspring (kits) were approximately 33 days old (133 days). Ergot alkaloids caused an initial transient decrease in feed consumption, but body weights were unaffected. The gestation period was significantly longer in females consuming 6 ppm or higher doses of ergot alkaloids compared to the controls. The number of females whelping varied significantly with 9 mink whelping each in the control and 3 ppm groups compared to 4 mink in the 6 ppm group and 1 in the 12 ppm group. The total number of kits whelped as well as the birth weight of kits was significantly lower than controls for females fed 3 ppm or higher doses of ergot alkaloids. Doses of 6 ppm or higher of ergot alkaloids had a significant effect on kit survivability with no kits surviving at 3 weeks of age. Plasma prolactin concentrations were significantly depressed for females in all groups fed diets containing ergot alkaloids. This study indicated that ingestion of ergot alkaloids at 3 ppm or higher resulted in reproductive toxicity in mink.

To my parents, Mr. and Mrs. Basu Dev Sharma

Who have given me all the happiness and support!!

ACKNOLEDGEMENTS

I finally have the opportunity to thank all the people who have helped, provided guidance, and supported me throughout my master's program. First of all, I would like to express my sincere gratitude to my major professor, Dr. Steven Bursian, who has been the best advisor I could have ever asked for. The years that I have worked with him, he has provided me with guidance, support, advice, friendship, and encouragement that has helped me to achieve my goals. Thank you very much Dr. Bursian. I would also like to thank all of my committee members: Dr Richard Aulerich, for his guidance, support, and friendship; Dr. Karen Chou for her guidance and advise; Dr. Jim Render for his assistance, advice and guidance; and Dr. Benjamin Yamini, also for his assistance, and advice.

I would like to thank Angelo Napolitano for his help throughout the trial period with animal care, breeding, blood collection, and necropsy. Angelo, without your help, all my work would have been impossible! My sincere thanks to Diane and Chris for all the help at the farm, especially taking good care of the mink. I would also like to thank Mr. Jerome Schafers for providing the ergot-contaminated wheat for my trial, Dr. George E. Rottinghaus for his help in analyzing the feed samples, and Dr. A. F. Parlow, NHPP and NIDDK for providing the assy kit for prolactin determination, and Dr. T. J. Reimers (Cornell University, NY) for performing the prolactin analysis.

Special thanks to friends like Breana Simmons and Elizabeth Molina for being there for me every time I have needed their help, and advice, and most of all for the friendship that they have given me. You all are great friends!! I would also like to thank

Kadir Kazilkiya for his help with statistical analysis and Rachel Mitchel for her technical help and friendship.

Finally, I would like to thank my parents, Mr. and Mrs. Basu Dev Sharma for their support and guidance throughout my master's program. They have given me the strength and courage that I needed to stay focussed on my goals, being so far away from home. Every time I cried on the phone due to homesickness, they have redirected my focus towards my priorities and helped me achieve my goals. Thank you mom and dad!!!!

Table of Contents

List of Tables.....vii

List of Figures.....viii

Introduction.....1

Literature Review..... 3

Materials and Methods..... 13

Results.....20

Discussion.....31

Conclusion.....42

References.....44

List of Tables

Table		Page
1	Specific ergot alkaloids detected in oats contaminated with *Claviceps purpurea*	15
2	Proximate analysis of basal mink diet	16
3	The effect of ergot alkaloids on adult female mink body weight change, reproductive parameters and plasma prolactin concentrations, and kit body weights and survivability	22
4	Effect of ergot alkaloids on whole blood parameters of adult female mink before breeding and after whelping	25
5	The effect of ergot alkaloids on serum chemistry parameters of adult female mink	26
6	The effect of ergot alkaloids on serum chemistry parameters of adult female mink prior to breeding	28
7	The effect of ergot alkaloids on serum chemistry parameters of adult female mink after breeding	29
8	The effect of ergot alkaloids on organ weights of adult female mink	30

List of Figures

Figure		Page
Figure 1	Structure of natural ergot peptide alkaloids	6
Figure 2	Effect of ergot alkaloids on feed consumption of adult female mink	21

Introduction

Ergot is a body produced by the fungi *Claviceps purpurea* that replaces the kernels in the mature grain head of a number of cereal grains and seed heads of grasses. The recorded history of ergot can be traced back to early as 600 BC when the structure was known as a noxious pustule in the ears of grain as referred to by an Assyrian tablet (Hofmann, 1978). In the Middle Ages, ergot was known as a poisonous contaminant of edible grain that caused human epidemics of ergotism in human beings in Europe (Hofmann, 1978). Ergotism was characterized by an extreme sensation in the extremities that was often followed by gangrene and loss of afflicted body parts (Hofmann, 1978; Flieger *et al.*, 1996). When the great epidemics of ergotism swept Europe in the Middle Ages, livestock production did not constitute as important a part of agriculture as it does now (Christensen, 1980). In the 1800s and 1900s, more incidents of animal toxicity due to ergot consumption were reported. Because animal production was becoming a major part of agriculture at this time, the relationship between exposure to ergot and animal toxicity was being studied more extensively (Christensen, 1980).

The first ergot alkaloid isolated was ergotoxine in 1906 and in 1918, ergotamine was isolated. Since then, some 40 ergot alkaloids, including isomeric forms, have been isolated from natural sources (Hofmann, 1978). Ergotamine was the first chemically pure ergot alkaloid that found widespread therapeutic use in obstetrics and internal medicine. Although the different ergot alkaloids were identified before 1920, it was not until 1934 that the common nucleus of all pharmacologically important ergot alkaloids was identified as lysergic acid (Hofmann, 1978).

Some of the animal species affected by the consumption of ergot alkaloids include cattle, sheep, swine, horses, poultry, and rats (Christensen, 1980). Grasses, such as fescue, and grains, such as wheat, barley, rye, and oats, that animals feed on are the major sources of ergot. Clinical signs in cattle, sheep, and poultry affected with ergot alkaloids include gangrene, reduced feed intake and weight gain, abortion, and acute convulsions. Studies in horses, sheep, and cattle indicated that consumption of ergot alkaloids resulted in reproductive effects such as anestrus, early embryo death, stillbirths, increased gestation length, and decreased prolactin concentration (Griffith *et al.*, 1978; McQueen, 1993; Schultze *et al.,* 1999).

Mice and other rodents have been the subjects of laboratory studies that examined the effects of ergot alkaloids on reproduction, lactation, and prolactin concentrations. Studies in rats showed reduced body weight gain, decreased reproductive rate and litter size, increased gestation length, decreased prolactin concentration, and variable effects on plasma/serum parameters (Tindal, 1956; Bennett *et al.*, 1977; Griffith *et al.*, 1978; Cross *et al.*, 1995; Paterson *et al.*, 1995; Cross, 1997; Browning *et al* 1997; Schultze *et al.*, 1999).

Ranch mink consume diets containing a certain portion of cereal grains (26% in the present study). Since ergot alkaloids can be contaminants of these grains, it is possible for mink to be exposed to these toxins. In the present study, adult female mink were administered diets containing different proportions of oats contaminated with ergot alkaloids for 133 days. The objectives of the study were to assess the effects of the ergot alkaloids on reproduction as well as on survivability and growth of the offspring.

Literature Review

Background

Mycotoxins are toxic chemicals produced by fungi, principally molds, growing on grains in the field or on grains, feed, or food in storage. Consumption of sufficient amounts of mycotoxins can result in poisoning, often called mycotoxicosis (Cheeke and Shull, 1985). The economic impact of reduced animal productivity, increased incidence of diseases due to immunosuppression, damage to vital organs, and interference with reproductive capacity is often greater compared to the impact caused by death due to mycotoxin poisoning (Genter *et al.,* 1999). Numerous studies have shown that mycotoxins such as deoxynivalenol, zearalenone, T-2 toxin, aflatoxins, fumonisins, moniliformin, and ergot alkaloids can be harmful to different animal species (McQueen, 1993; Woloshuk, 1997).

Ergot is a body produced by the fungi *Claviceps purpurea* that replaces the kernels in the mature grain head or grass seed heads. The resulting hard, dark-colored, horn-like masses are called sclerotia. The word ergot is derived from the French word for "cockspur", a description of the infected curved grain head (Raymond, 1995). During the spring, the fungus inside the sclerotia forms stromas with spores that are then spread to other kernels by insects, wind, or rain, resulting in infection of the plant. Ergot infects cereal grains such as barley, wheat, oats, and rye as well as ryegrass (a cross-pollinated grain), wheatgrass, bluegrass, and fescue (McQueen, 1993).

History

During the Middle Ages, ergot caused epidemics of ergotism in Europe that killed thousands of people who consumed bread made from ergot-contaminated rye (Hofmann,

1978). Affected individuals had convulsions and displayed *Ergotismus gangraenosus*, also known as "St. Anthony's fire", "holy fire" or "ignis sacer". This disease was particularly characterized by an extreme burning sensation in the extremities that was often followed by gangrene and loss of the afflicted body part (Flieger *et al.*, 1996).

When the ergot epidemics broke out in Europe during the Middle Ages, livestock production did not constitute an important part of agriculture as it does now (Christensen, 1980), and thus little was known about the effects of ergot on animals. During the 1800s and 1900s, more incidents of animal toxicity due to ergot consumption were reported. Typical clinical signs reported in livestock species included swollen joints, lameness, elevated body temperature, numbness, depression, reduced feed intake, reduced body weight gain, reduced milk production, gangrene, decreased prolactin concentration, and direct or indirect reproductive effects ranging from anestrus to early embryonic death (McQueen, 1993).

Alkaloid isolation and structure

During the late 1800s, because animal production was becoming a major part of agriculture, scientists started performing controlled studies on ergot and its effects. In the early 1900s, a number of different alkaloids were isolated from ergot. In 1906, the first ergot alkaloid, ergotoxine, was identified, followed by the isolation of ergotamine in 1918 (Hofmann, 1978). Since then, approximately 40 ergot alkaloids have been identified. During the process of ergot alkaloid isolation, the common nucleus of all ergot alkaloids was characterized and subsequently named lysergic acid. The major toxic groups of ergot alkaloids include the ergotamine group (ergotamine and ergosine), the ergoxine group (ergostine), and the ergotoxine group (ergocristine, ergocornine, and α-

and β-ergokryptine) (Rutschmann and Stadler, 1978; Cheeke and Shull, 1985). The ergot structures are presented in Figure 1.

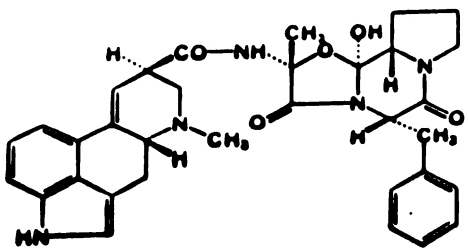
General effects of ergot alkaloids

Ergot alkaloids possess a divergent spectrum of central and peripheral pharmacodynamic actions. The central actions include inhibition of vasomotor tone, heart rate, and circulatory reflexes. The peripheral actions include the two most important properties of ergot alkaloids, vasoconstriction and uterotonic effects (increase in uterine motor activity). The other properties of ergot alkaloids include adrenolytic effects (α-adrenergic blocking action) and decreased prolactin secretion (Boissier, 1978).

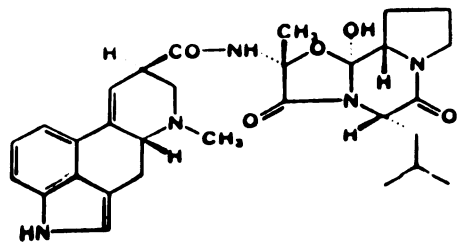
For centuries, ergot alkaloids have been known to be toxic to both animals and human beings. Even though ergot alkaloids were used as remedies for complications during childbirth (control of postpartum hemorrhage) and migraine headaches, their toxic effects were soon studied by scientists. In humans, ergot alkaloid toxicity is manifested in cardiovascular effects (vasospasm, gangrene), central nervous system effects (headache, psychosis, hallucinations), gastrointestinal effects (ulceration, hepatotoxicity), and reproductive effects (abortion, inhibition of lactation). Ergot alkaloids cause similar effects in animals including hallucinations, gastric ulceration, abortion, embryotoxicity, inhibition of implantation and lactation, and uterine tumors (Griffith *et al.*, 1978).

Ergot alkaloids exert their effects in different ways. Ergotamine binds strongly to tissues (liver, kidneys, lungs), which accounts for the persistence of biological effects well after the parent drug or metabolites can no longer be detected in plasma (the half-life of ergotamine is about 2 hours) (Rall and Schleifer, 1980; Silberstein, 1997). Alkaloids in the ergotoxine group, such as ergocristine, ergocornine, and ergokryptine, are the most

Ergotamine group

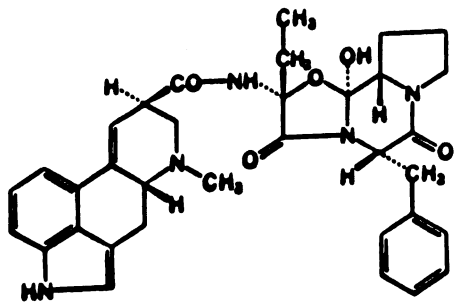


Ergotamine



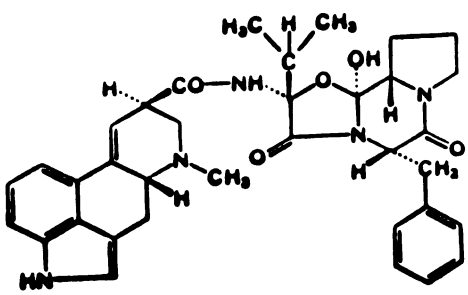
Ergosine

Ergoxine group

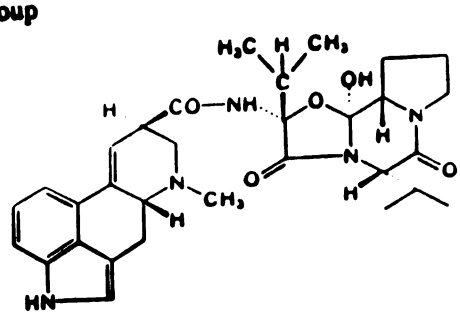


Ergostine

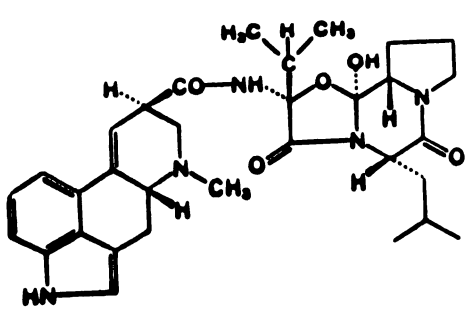
Ergotoxine group



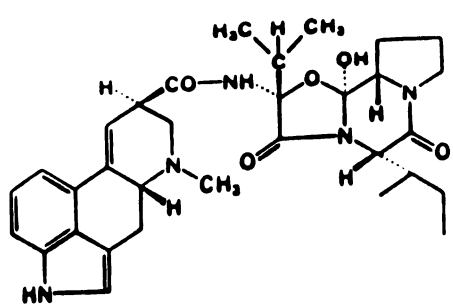
Ergocristine



Ergocornine



α-Ergokryptine



β-Ergokryptine

Figure 1. Structure of natural ergot peptide alkaloids

effective in terms of inhibition of implantation compared to the other ergot alkaloids (Rutschmann and Stadler, 1978). Alkaloids of the ergotoxine group also act via the hypothalamus and pituitary to inhibit prolactin secretion (Fluckiger *et al.*, 1976).

The major route of exposure to ergot alkaloids for livestock is through grazing of ergot-infected or endophyte-infected plants. Tall fescue infected with the endophytic fungus *Acremonium coenophialum* has been shown to be a source of ergot alkaloids, and these are believed to cause the clinical signs of fescue toxicosis that are characterized by decreased feed intake, reduced blood flow to the periphery, decreased conception rates, and decreased serum prolactin concentrations (Samford-Grigsby *et al.*, 1997). The clinical signs of ergot toxicity and fescue toxicity are similar and studies have reported ergot alkaloids to be a major contributor of fescue toxicosis (Piper *et al.*, 1997; Browning, 1998). According to Browning *et al.* (1998), ergot alkaloids such as ergovaline and ergosine are found in high concentrations in cases of endophyte-infected fescue toxicosis, whereas ergotamine and ergonovine are found in lower concentrations.

Effect on feed intake

Consumption of ergot alkaloids has been reported to cause variable effects on feed intake in different animals such as rats and cattle. Jackson *et al.* (1989) reported no decrease in feed consumption when rats were fed diets containing 1.25 mg ergonovine, ergocryptine or ergotamine/kg feed. In a study by Peters-Volleberg *et al.* (1996), rats fed diets containing ergometrine (2, 10, 50 or 250 mg ergometrine/kg feed) had numerically lower feed intake compared to controls fed *ad lib*, but the effect was not statistically significant. However, rats fed diets consisting of 50% endophyte-infected seed containing

5 mg ergovaline and 1 mg ergine/kg feed for 28 days had significantly lower feed intake compared to controls (Piper *et al.,* 1997).

Weinstein *et al.* (1999) performed a subacute feeding trial in which mink were administered ergot alkaloids. In that particular study, ground sclerotia containing 100 ppm ergot alkaloids (8% ergosine, 14% ergotamine, 17% ergocryptine, and 39% ergocristine) were added to the basal diet. Mink fed 6.25, 12.5, or 25 ppm ergot alkaloids for 30 days showed an initial feed refusal, but no other clinical signs of toxicity.

Effect on body weight

Consumption of diets containing ergot alkaloids was found to be related to reduced body weight gains in animals. The body weights of rats fed diets containing 2, 10, 50, or 250 mg ergometrine/kg feed for 4 weeks were lower than those of the controls, but the dose-response relationship was not significant (Peters-Volleberg *et al.,* 1996). In another study (Piper *et al.,* 1997), rats fed a diet consisting of 50% endophyte-infected seed containing 5 mg ergovaline and 1 mg ergine/ kg feed for 28 days had a significant reduction in body weight gain compared to controls. Numerous studies have indicated that consumption of endophyte-infected fescue causes a decrease in body weight of cattle (Cross *et al.,* 1995; Paterson *et al.,* 1995).

Effect on gestation

Consumption of endophyte-infected tall fescue is reported to cause an increase in gestation length in animals. For example, consumption of endophyte-infected tall fescue prolonged gestation in mares in excess of the normal range of 335 to 345 days (Porter and Thompson, 1992), while in another study, mares consuming endophyte-infected fescue

grass had gestation length increased by an average of 27 days compared to mares on a control diet (Cross, 1997).

Effect on reproduction

Consumption of ergot alkaloids is known to cause reproductive failure in animals, both in the laboratory and in the field. Ergot alkaloids can induce abortion, stillbirths, agalactia, malnutrition, and deformations of the progeny (Griffith *et al.*, 1978). These kinds of reproductive effects have been seen in rats, mice, livestock species, and monkeys.

Most of the laboratory studies on the reproductive effects of ergot alkaloids have been performed using rats and mice. A single subcutaneous injection of ergotoxine (0.1 – 1.0 mg/rat) given to rats during early gestation terminated pregnancy within 1-3 days (Shelesnyak, 1954, 1955). In another study, rats were administered 2 doses of 0.5 mg ergotamine or ergotoxine/kg body weight by intraperitoneal injection 10 days apart. With both compounds, there were unsuccessful matings, resorbed fetuses at early and late stages of pregnancy, and stillbirths (Sommer and Buchanan, 1955).

Cattle exposed to endophyte-infected tall fescue develop reproductive problems. According to Porter and Thompson (1992), reduced reproductive efficiency (lower pregnancy rates, delayed conception) in cattle grazed on endophyte-infected fescue is a part of a syndrome often referred to as fescue summer toxicosis or summer slump. The authors reported that the calving rate for cows grazing on low (0 to 5% infected) endophyte-infected fescue was 86% compared to 67% for cows grazing on high (80 to 90% plants infected) endophyte-infected fescue. Similarly, 96% of beef heifers

consuming low endophyte-infected fescue conceived compared to 55% for heifers consuming high endophyte-infected fescue (Schmidt *et al.*, 1986).

Besides reducing reproduction rates in animals, consumption of ergot alkaloids has been reported to cause a decrease in body weight and an increase in mortality of the offspring. Rats given intraperitoneal injections of 0.5 mg ergotamine/kg body weight or 0.5 mg ergotoxine/kg body weight had pups with significantly lower birth weights compared to the pups in the control group (Sommer and Buchanan, 1955). In the same experiment, the authors reported higher mortality of pups in the ergotamine-treated rats compared to the control group. On the 16^{th} day of the trial, only 23 pups were remaining of the 37 born.

Effect on prolactin

Decreased serum prolactin concentrations due to consumption of ergot alkaloids and/or endophyte-infected tall fescue have been reported in cattle. Serum prolactin concentrations were significantly lower in steers fed 370 ppb ergovaline for 30 days compared to the controls (Samford-Grigsby *et al.*, 1997). In another study, cattle infused with an average dose of 23.8 μg/kg body weight of ergotamine and ergonovine had decreased serum prolactin concentrations (Browning *et al.*, 1997). Bolt *et al.* (1982) reported decreased serum prolactin concentrations in ewes consuming endophyte-infected fescue.

Laboratory studies have shown that consumption of ergot alkaloids reduces serum prolactin concentrations in rats. Rats fed diets containing 5 mg ergovaline and 1 mg ergine/kg feed for 28 days had reduced serum prolactin concentrations compared to controls (Piper *et al.*, 1997). Similar to this study, rats given 20 mg ergometrine/kg body

weight had a 73% reduction in serum prolactin concentrations when measured 6 hours after the fifth dose (Shaar and Clemens, 1972).

Effect on clinical chemistries

Consumption of ergot alkaloids has been reported to have a varied effect on plasma serum parameters in different animal species such as cattle (Schultze *et al.*, 1999) and rats (Peters-Volleberg *et al.*, 1996). Cattle pastured on endophyte-infected tall fescue for 7 months had a greater albumin/globulin (A/G) ratio compared to the controls whereas alkaline phosphatase activity was lower in the serum of cattle grazing on endophyte-infected fescue (Schultze *et al.*, 1999). The authors explained that the increase in the A/G ratio was related to a decrease in the globulin concentration whereas the decrease in alkaline phosphatase activity was due to decreases in the intestinal and bone isozyme activities. Alanine aminotransferase is an enzyme within the cytoplasm of hepatic parenchymal cells. Increase of this enzyme activity in the plasma/serum after consumption of endophyte-infected fescue occurs due to leakage from injured hepatocytes, but little is known regarding the clinical significance of decreased activity of this enzyme and others. Possible explanations for the decrease in serum enzyme activities (alkaline phosphatase and alanine aminotransferase) and globulin concentration are reduced intake of feed, nutrient deficits of vitamins, protein or minerals, decreased enzyme production due to decreased cell proliferation, or increased clearance of enzymes via liver or kidneys (Schultze *et al.*, 1999). Rats fed 50 and 250 mg ergometrine/kg feed had significantly lower plasma glucose concentrations compared to controls (Peters-Volleberg *et al.*, 1996). The authors explained that although ergometrine affects

carbohydrate metabolism, the mechanism of action for the decrease in glucose is not known.

Effect on tissue weight and morphology

The effect of ergot alkaloids on tissue weight has been reported in rats fed ergometrine at doses of 2, 10, 50 or 250 mg/kg diet for 4 weeks (Peters-Volleberg *et al.*, 1996). The authors reported an increase in absolute and relative heart weight (250mg/kg), absolute liver weight (10 and 250 mg/kg), and relative liver weight (2, 10, 50, 250 mg/kg). In the same study, the authors reported histological changes only in the liver. Relatively large and swollen hepatocytes were observed with cytoplasmic staining in the central part of the cell and at the cellular wall, which is considered typical of glycogen storage. Although this was the only effect seen in the liver, there was no evidence of ergometrine-induced hepatocellular degeneration or necrosis or other liver abnormalities at the above doses. The kidneys showed moderate to severe mineralization in the interocorticomedullary area, but this occurred in all groups including the controls, and was thus not an indication of a treatment effect.

Study objective:

Animal health problems due to the consumption of ergot alkaloids have been studied extensively during the last several years. With many scientific studies focusing on this problem, livestock producers have become more knowledgeable about the potential problems posed by ergot alkaloids. Incidents of ergot toxicity continue to be reported in the United States. In 1990 and 1991, ergot bodies were found in unusually high levels in mature grasses throughout the United States with up to 50% or more of individual seeds being replaced by ergot bodies. In 1992, heavy ergot infestation of grass seed heads was

reported in Illinois (McQueen, 1993). Bennet-Wimbush and Loch (1997) reported that approximately 58% of the 15 million hectares of tall fescue grown in the United States was infected with the endophyte fungus *Acremonium coenophilum*.

A portion of the ranch mink diet is cereal, thus there is the potential for mink to consume toxins found in plant material. Previous studies from this laboratory have indicated that mink are susceptible to a number of different mycotoxins. T-2 toxin (0.25 to 4.0 mg/kg diet) caused a decrease in feed consumption and body weight of mink (Aulerich *et al.*, 1987). Reproductive effects such as reduced whelping, litter size, and kit body weight and increased gestation length were reported in mink fed zearalenone (20 mg/kg feed) (Bursian *et al.*, 1992), and fumonisins (115 to 254 mg/kg feed) (Powell *et al.*, 1996). In a sub-acute study, 9-month old mink fed 25 mg ergot alkaloids/kg feed (8% ergosine, 14% ergotamine, 17% ergocornine, 23% ergocryptine, and 39% ergocristine) experienced an initial decrease in feed consumption, but there were no clinical signs of toxicity, no gross or histologic lesions, and no alterations in hematologic or blood chemical values compared to the controls (Weinstein *et al.*, 1999). Since the sub-acute study in mink by Weinstein *et al.* (1999) was a 30-day trial and revealed no clinical signs of toxicity, the present study was performed to examine the reproductive effects in mink consuming ergot alkaloid-contaminated feed over a longer period of time (133 days).

Materials and Methods

Experimental design:

Forty-eight female, non-sibling, pastel mink were randomly allocated to 4 dietary treatment groups of 12 mink each. Mink were housed individually in suspended wire

cages (76 cm L x 61 cm W x 46 cm H) in an enclosed room at the Michigan State University (MSU) Experimental Fur Farm. A wooden nest box (38 cm L x 28 cm W x 27 cm H), bedded with aspen shavings and excelsior (wood wool), was attached to the outside of each cage. The room temperature was maintained above 0°C with thermostatically controlled electric heaters. Ceiling vents and a wall fan provided ventilation. A time clock was used to provide light that mimicked the natural photoperiod. The mink were acclimated to this environment, being fed a standard ranch mink diet for 7 days prior to the beginning of the trial on February 15, 1999. Feed and water were provided *ad libitum*.

Diet preparation:

Oats contaminated with *Claviceps purpurea* were received from Jerome Schafers (Miller, South Dakota) on January 27, 1999 and ground at the MSU Experimental Fur Farm. A sample of the oats was analyzed for ergot alkaloids by Dr. G. E. Rottinghaus of the Veterinary Medical Diagnostic Laboratory, University of Missouri (Columbia, MO). The sample contained 109.5 ppm total ergot alkaloids. Specific alkaloids are presented in Table 1.

The experimental diets were prepared by adding ground oats to the basal mink diet composed of 11% raw eggs, 5% fishmeal (menhaden), 9% spray-dried chicken liver, 26% duck by-products, 23% water, and 0.12 mg d-biotin/kg diet). The remaining 26% of the diet consisted of a mixture of uncontaminated and contaminated oats such that the treatment diets contained targeted concentrations of 0.0 (control), 3.0, 6.0, or 12.0 ppm ergot alkaloids. Three samples of the control diet were submitted to Litchfield Analytical Service (Litchfield, MI) for proximate analysis (Table 2).

Table 1. Specific ergot alkaloids detected in oats contaminated with *Claviceps purpurea*		
Ergot alkaloid	Concentration (ppm)	% of total ergot alkaloids
Ergosine	7.5	6.8
Ergotamine	14.1	12.9
Ergocornine	16.1	14.7
Ergocryptine	17.5	16.0
Ergocristine	54.3	49.6

Table 2. Proximate analysis of basal mink diet		
Parameters	Wet basis	Dry basis
Moisture (%)	51.50	-
Dry matter (%)	48.50	-
Fat (%)	7.81	16.10
Crude protein (%)	12.75	26.28
Crude fiber (%)	2.52	5.20
Ash (%)	3.83	7.90
TDN (%)	41.96	86.51
Calcium (%)	0.80	1.66
Phosphorus (%)	0.58	1.20
Potassium (%)	0.38	0.79
Magnesium(%)	0.09	0.18
Sodium (%)	0.21	0.43
Iron (ppm)	413.00	851.00
Manganese (ppm)	29.00	60.00
Copper (ppm)	5.00	11.00
Zinc (ppm)	47.00	97.00

Determination of body weight, feed consumption, and collection of blood samples

Adult mink were weighed on days 1, 7, and 35 of the trial as well as at whelping (approximately 81 days on trial), and at necropsy (approximately 133 days on trial).

Determination of feed consumption was conducted from day 1 through day 7 of the trial. On day 10, blood samples were collected from mink in the control and the 12 ppm ergot alkaloid groups by jugular venipuncture. Mink were anesthetized with 0.3 ml of ketamine hydrochloride (100 mg/ml; Fort Dodge Animal Health, Fort Dodge, IA) administered intramuscularly prior to blood collection. Blood was collected in 2 microhematocrit capillary tubes (Becton Dickinson, Franklin Lakes, NJ), 1 2-ml Vacutainer® tube (Becton Dickinson, Franklin Lakes, NJ) containing EDTA and 1 1-ml Vacutainer® tube with no additive. The microhematocrit capillary tubes were centrifuged in an IEC MB microhematocrit centrifuge (International Equipment Company, Boston, MA), and hematocrits were determined with an IEC MB microcapillary reader. The Vacutainer® tubes with no additive were submitted to the MSU Animal Health Diagnostic Laboratory for determination of clinical chemistries. The Vacutainer® tubes containing EDTA were used to determine the hemoglobin concentration using a kit (Sigma Diagnostics, St. Louis, MO).

On May 20, 1999, (94 days on trial) blood samples were taken from the control females and from the females fed 12.0 ppm ergot alkaloids by jugular venipuncture as described previously. In addition to the blood collected for determination of hematocrit,

hemoglobin concentration and clinical chemistries, blood was also collected from the females in all of the treatment groups for determination of plasma prolactin concentrations. The latter plasma samples were shipped on dry ice to the Cornell University Veterinary Diagnostic Laboratory (Ithaca, NY) for determination of prolactin concentrations.

Breeding and whelping

On March 1, 1999, mink breeding began. Twelve untreated males were randomly selected from the MSU Experimental Fur Farm herd to breed with the 48 females on treatment. The males were housed in the same room as the females. During breeding, a female was placed in a male's cage. If the male mounted the female, the animals were left undisturbed until they separated. Upon separation, a vaginal aspiration was taken from the female and examined under a light microscope. If motile sperm were observed, the mating was assumed to be successful. If the mating was not successful or if the pair was not compatible, the female was tried with another male either the same day or within the next 2-4 days. Mink that had a successful mating were mated again either the next day or 8 days later. Breeding was completed on March 27, 1999.

During the whelping period, the nest boxes were checked daily for kits. Newborn kits were counted, sexed, and weighed. The kits were observed daily for mortality until they were 3 weeks old at which time body weights were determined again.

Necropsies

Adult female mink and kits were necropsied after the kits were 3 weeks old. Necropsies were performed on 4 different dates; May 20, May 28, June 7, and June 28, 1999. Mink that did not whelp were necropsied last to assure that they were in fact not

pregnant. Mink were euthanized by exposure to carbon dioxide. The liver, brain, heart, lungs, adrenal glands, kidneys, and spleen were removed, weighed, and samples were fixed in 10% neutral-buffered formalin. Fixed tissues were prepared for histologic examination using routine histotechnological methods. The tissues were mounted in paraffin, sectioned (6 μm), stained with hematoxylin and eosin, and examined using a light microscope. In addition, the uteri of the mink that did not whelp were examined for resorbed fetuses.

Statistical analysis

All statistical analyses were performed using SAS (SAS 1999, 2000). Feed consumption, body weight, hemoglobin concentration, hematocrit, and serum chemistry were analyzed using analysis of variance (ANOVA) involving the factor treatment with repeated measurements on individual animals, over a third factor day/date. Since measurements of feed consumption, adult body weight, hemoglobin concentration, hematocrit, and serum chemistries were taken at adjacent time periods, the repeated measure procedure was used to analyze the data. Organ weights, plasma prolactin concentration, gestation period of adult females, and kit body weight were analyzed using the general linear model (PROC GLM) procedure. Chi-square analysis was used to test the total number of kits whelped and the number of kits born alive in each treatment. Treatment group means were reported as the least square mean plus or minus the standard error. If there was a significant treatment and day/date interaction, the means were analyzed separately for each day/date. Differences between the treatment groups were considered statistically significant based on a Type I error rate of 0.05.

Results

Feed consumption

Feed consumption of adult female mink fed diets containing different concentrations of ergot alkaloids was depressed for the first 3 days of the trial compared to controls. On day 4 of the trial, the mink in the 6 and 12 ppm ergot alkaloid treatment groups were still consuming significantly less feed compared to controls, but by day 7, all mink were consuming a similar amount of feed (Figure 2).

Body weights

The consumption of ergot alkaloids had no significant effect on body weight of adult female mink. All treatment groups lost a similar percentage of weight during the 19-week trial (Table 3).

Mating and whelping

Consumption of ergot alkaloids had no deleterious effect on mating success, although females in the control group had earlier first matings compared to females in all ergot alkaloid groups (8.5 days after breeding began for controls vs. 9.6, 12.6 and 11.0 days for mink in the 3, 6 and 12 ppm ergot alkaloid groups respectively). However, the consumption of ergot alkaloids at all concentrations (3, 6 and 12 ppm) caused a dose-dependent decrease in the number of mink whelping (Table 3).

Gestation

The gestation period (from the date of the last confirmed mating to whelping) of mink fed ergot alkaloids was significantly increased by consumption of 6 ppm ergot alkaloids compared to controls (Table 3). The single female in the 12 ppm group that

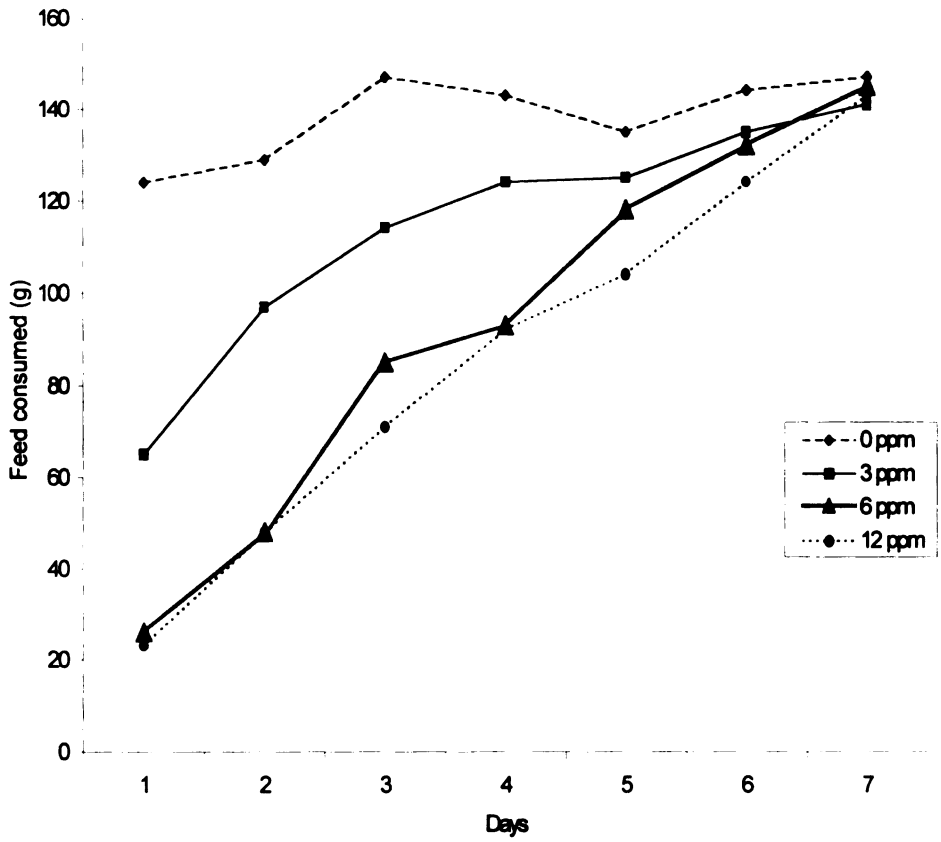


Figure 2. Effects of ergot alkaloids on feed consumption of adult female mink

Table 3. The effect of ergot alkaloids on adult female mink body weight change, reproductive parameters, and plasma prolactin concentrations, and kit body weights and survivability.

Parameters	Treatment			
	Control	3 ppm	6 ppm	12 ppm
% body weight decrease*	15.52 ±3.54 (12)	8.24 ± 3.55 (12)	13.29 ± 3.48 (12)	8.18 ± 3.83 (11)
# females bred / total # females	11/12	12/12	12/12	11/12
# females whelping / # females bred	9/11	9/12	4/12	1/11
Gestation (days)*	47 ± 2.84[a] (9)	52 ± 2.84[a] (9)	60 ± 4.26[b] (4)	62 (1)
Plasma prolactin concentration (ng/ml)*	51.1 ± 4.15[a] (12)	18.3 ± 5.09[b] (8)	12.2 ± 4.16[b] (12)	11.8 ±4.55[b] (10)
Total # kits whelped	44[a] (9)	36[b] (9)	16[c] (4)	3[d] (1)
% live kits whelped	75[a]	33[b]	6[c]	0
Kit birth weight (g)*	11.1 ± 0.40[a] (33)	8.8 ± 0.70[b] (12)	9.7 (1)	-
% live kits at 3 weeks	85	83	0	0
Kit weight at 3 weeks (g)*	113.0 ± 3.77[a] (28)	69.5 ± 6.31[b] (10)	_	_

*Data presented as mean ± standard error. Numbers in parentheses refer to sample size. Means with different superscripts in the same row are significantly different from one another at $p<0.05$.

whelped had a gestation period 2 days longer than the average gestation period of mink in the 6 ppm group. During necropsy, 1 female in the 3 ppm ergot alkaloid group, and 2 females in the 6 ppm ergot alkaloid group were discovered to have live fetuses. The female in the 3 ppm ergot alkaloid group had 4 live fetuses at 78 days of gestation and the 2 females in the 6ppm ergot alkaloid group had 5 and 6 live fetuses at 68 days and 69 days of gestation, respectively. Besides the females that had live fetuses in the uterus, there were 3 females in the 12 ppm ergot alkaloid group that had 8, 6, and 2 fetuses in the process of being resorbed at 65, 64, and 56 days of gestation respectively.

Prolactin

Consumption of ergot alkaloids at 3 ppm or higher had a significant effect on plasma prolactin concentrations compared to the control group. The mink in the control group had plasma prolactin concentrations over 4 times higher than plasma prolactin concentrations in mink in the 12 ppm ergot alkaloid group (Table 3).

Kit survivability

Consumption of ergot alkaloids at all concentrations caused a significant decrease in the total number of kits whelped. The percent of kits born alive was significantly decreased in all 3 groups fed ergot alkaloids. By 3 weeks of age, the kits in the 6 ppm ergot alkaloid group had all died. Percent morality of the control and 3 ppm ergot alkaloid groups was 15% and 17%, respectively, at 3 weeks of age (Table 3). In addition to decreases in litter size, stillborn kits were whelped by 2 females in the 3 ppm ergot alkaloid group and 1 female each in the 6 ppm and 12 ppm ergot alkaloid groups at 58, 53, 84 and 62 days of gestation respectively. These kits had congenital deformities (enlarged head, and small body).

Kit body weight

The birth weight of kits in the 3 ppm ergot alkaloid was significantly less than the birth weight of control kits. At 3 weeks of age, the control kits were 43% heavier than the kits in the 3 ppm ergot alkaloid group (Table 3).

Hemoglobin concentration and hematocrit

Hemoglobin concentration and hematocrit were determined prior to breeding and after whelping in the adult females in the control and 12 ppm ergot alkaloid groups only. Since there was a significant treatment by date interaction, the data were presented for each blood collection date. There were no significant differences in the hemoglobin concentration or hematocrit between the control and 12 ppm ergot alkaloid groups at either time period (Table 4).

Serum chemistries

Serum chemistries were assessed only in control females and females in the 12 ppm ergot alkaloid group prior to breeding and after whelping. Consumption of ergot alkaloids had a variable effect on serum chemistry parameters. There were no significant treatment by date interactions or treatment-related effects on concentrations of sodium, anion gap, total protein, albumin, and globulin concentrations, albumin/ globulin ratio, concentrations of total bilirubin and creatinine, activities of alkaline phosphatase and aspartate aminotransferase, concentrations of calcium, magnesium, and blood urea nitrogen, or osmolality. However, consumption of 12 ppm ergot alkaloids caused a significant decrease in alanine aminotransferase activity and cholesterol concentration compared to controls (Table 5).

Table 4. Effect of ergot alkaloids on whole blood parameters of adult female mink before breeding and after whelping*.				
Parameter	Treatment			
	Pre-breeding (2/25/99)		Post-whelping (5/20/99)	
	Control	12 ppm	Control	12 ppm
Hemoglobin	15.02 ± 0.42 (12)	15.56 ± 0.44 (11)	17.63 ± 0.42 (12)	17.57 ± 0.44 (11)
Hematocrit	46.61± 1.04 (12)	49.27± 1.08 (11)	44.93± 1.08 (12)	43.31± 1.08 (11)
*Data presented as mean ± standard error. Hemoglobin is expressed as g/dL. Hematocrit is expressed as percentage of packed red blood cell volume. Numbers in parentheses refer to sample size.				

Table 5. The effect of ergot alkaloids on serum chemistry parameters of adult female mink*			
Parameter	Units	Treatment	
		0 ppm	12 ppm
Sodium	mmol/l	155.42 ± 0.470 (12)	154.64 ± 0.475 (11)
Anion gap	mmol/l	16.58 ± 0.728 (12)	15.79 ± 0.746 (11)
Total protein	g/dl	5.85 ± 0.128 (12)	5.72 ± 0.129 (11)
Albumin	g/dl	3.35 ± 0.074 (12)	3.26 ± 0.075 (11)
Globulin	g/dl	2.49 ± 0.068 (12)	2.41 ± 0.069 (11)
Albumin/ Globulin ratio		1.35 ± 0.027 (12)	1.37 ± 0.028 (11)
Total bilirubin	mg/dl	0.12 ± 0.023 (12)	0.18 ± 0.024 (11)
Creatinine	mg/dl	0.87 ± 0.035 (12)	0.80 ± 0.036 (11)
Alkaline phosphatase	IU/l	51.70 ± 6.693 (12)	42.76 ± 6.829 (11)
Alanine aminotransferase	IU/l	197.67 ± 19.686 [a] (12)	121.75 ± 20.046 [b] (11)
Aspartate aminotransferase	IU/l	95.88 ± 6.614 (12)	107.83 ± 6.779 (11)
Calcium	mg/dl	9.62 ± 0.140 (12)	9.47 ± 0.142 (11)
Cholesterol	mg/dl	257.00 ±10.089 [a] (12)	196.46 ± 11.006 [b] (11)
Magnesium	mg/dl	2.53 ± 0.067 (12)	2.59 ± 0.068 (11)
Blood urea nitrogen	mg/dl	20.62 ± 0.912 (12)	18.59 ± 0.938 (11)
Osmolality	mOs/kg	324.71 ± 1.059 (12)	322.20 ± 1.072 (11)
*Data presented as mean ± standard error. Numbers in parentheses refer to sample size. Means with different superscripts are significantly different within the row ($p < 0.05$)			

Before breeding, consumption of 12 ppm ergot alkaloids caused an increase in chloride and glucose concentrations and a decrease in carbon dioxide concentration. After whelping, there were significant decreases in chloride, glucose and iron concentrations, sodium/potassium ratio, and amylase activity in the animals fed 12 ppm ergot alkaloids. Conversely, consumption of 12 ppm ergot alkaloids caused significant increases in potassium, carbon dioxide, and phosphorus concentrations and creatnine kinase activity after whelping (Table 6 and 7).

Organ weights and histopathology

Consumption of up to 12 ppm ergot alkaloids had no significant effect on weights of brain, liver, spleen, kidney, heart, and adrenals in adult female mink (Table 8).

Upon histological examination, the livers of females in all treatment groups had variable amounts of intracytoplasmic glycogen and lipid. The medullary tubules of the kidneys appeared to have some intracytoplasmic lipid, and the amount of cortical epithelial vacoulation was variable. There appeared to be a trend towards increased vacoulation of the renal epithelial cells with increasing dose, but there was considerable variability. In the spleen, the amount of extramedullary hematopoiesis with the presence of megakaryocytes was variable. The thickness of the marginal zone of the spleen was also variable. In some mink, there was an irregular deposit of amorphous, eosinophilic material in the white pulp. There appeared to be a prominence of white pulp in the spleen of mink fed the highest concentration of ergot alkaloids.

Table 6. The effect of ergot alkaloids on serum chemistry parameters of adult female mink prior to breeding (2/25/99)*			
Parameters	Units	Treatment	
		0 ppm	12 ppm
Potassium	mmol/l	4.40 ± 0.137 (12)	4.09 ± 0.137 (12)
Chloride	mmol/l	117.61 ± 0.475 [a] (12)	119.52 ± 0.475 [b] (12)
Total carbon dioxide	mmol/l	25.31 ± 1.046 [a] (12)	21.27 ±1.046 [b] (12)
Sodium/ potassium ratio		35.42 ± 0.925 (12)	38.00 ± 0.925 (12)
Amylase	U/l	59.50 ± 5.108 (12)	62.08 ± 5.108 (12)
Creatnine kinase	IU/l	559.25 ± 238.100 (12)	575.00 ± 238.100 (12)
Glucose	mg/dl	122.17 ± 6.545 [a] (12)	142.83 ± 6.545 [b] (12)
Phosphorus	mg/dl	4.08 ± 0.303 (12)	3.45 ± 0.303 (11)
Iron	ug/dl	142.92 ± 12.826 (12)	150.25 ± 12.826 (11)
*Data presented as mean ±standard error. Numbers in parentheses refer to sample size. Means with different superscripts within the same row and time period are significantly different ($p < 0.05$).			

Table 7. The effect of ergot alkaloids on serum chemistry parameters of adult female mink after whelping (5/20/99)*			
Parameters	Units	Treatment	
		0 ppm	12 ppm
Potassium	mmol/l	4.75 ± 0.137[a] (12)	5.48 ± 0.142[b] (11)
Chloride	mmol/l	120.69 ± 0.475[a] (12)	119.22 ± 0.493[b] (11)
Total carbon dioxide	mmol/l	23.34 ±1.046[a] (12)	27.42 ±1.092[b] (11)
Sodium/ potassium ratio		32.92 ± 0.925[a] (12)	28.55 ±0.956[b] (11)
Amylase	U/l	88.00 ± 5.108[a] (12)	73.19 ± 5.217[b] (11)
Creatnine kinase	IU/l	399.58 ± 238.100[a] (12)	1859.44 ± 248.450[b] (11)
Glucose	mg/dl	109.08 ± 6.545[a] (12)	83.96 ± 6.826[b] (11)
Phosphorus	mg/dl	5.14 ± 0.303[a] (12)	6.49 ± 0.317[b] (11)
Iron	ug/dl	217.25 ±12.826[a] (12)	162.64 ±13.396[b] (11)
*Data presented as mean ±standard error. Numbers in parentheses refer to sample size. Means with different superscripts within the same row and time period are significantly different ($p < 0.05$).			

Table 8. Effect of ergot alkaloids on organ weights (g) of adult female mink*				
Organ	Treatment			
	Control	3 ppm	6 ppm	12 ppm
Brain	8.61 ± 0.17 (12)	8.81 ± 0.17 (12)	8.85 ± 0.17 (12)	8.54 ± 0.17 (12)
Liver	38.05 ± 1.98 (12)	35.88 ± 1.98 (12)	35.62 ± 1.98 (12)	32.77 ±1.98 (12)
Spleen	4.15 ± 0.83 (12)	3.16 ± 0.83 (12)	3.86 ± 0.83 (12)	3.05 ± 0.83 (12)
Kidneys	5.73 ± 0.21 (12)	5.91 ± 0.21 (12)	5.69 ± 0.21 (12)	5.78 ± 0.21 (12)
Heart	5.99 ± 0.17 (12)	6.39 ± 0.17 (12)	6.08 ± 0.17 (12)	6.32 ± 0.17 (12)
Adrenal glands	0.046 ± 0.004 (12)	0.048 ± 0.004 (12)	0.044 ± 0.004 (12)	0.046 ± 0.004 (12)
*Data presented as mean ± standard error. Numbers in parentheses refer to sample size.				

Discussion

In the present study, consumption of ergot alkaloids caused a transitory decrease in feed consumption of adult female mink. Ergot alkaloids have been reported to have a variable effect on feed consumption in different animal species. Rats fed a diet containing 5 ppm ergovaline and 1 ppm ergine for 28 days had a significant decrease in feed consumption (Piper *et al.*, 1997). However, rats given intraperitoneal injections of 0.5 mg ergotamine/kg body weight consumed as much food as prior to the injections (Sommer and Buchanan, 1955). McQueen (1993) hypothesized that a decrease in feed intake in cattle consuming ergot alkaloids could have been related to heat stress during warm weather. Since our study was performed during the winter in a minimally heated facility, absence of excessive heat could have been the reason why there was not an ergot alkaloid-induced effect on feed intake. It is also possible that the concentrations of ergot alkaloids (either singly or in combination) used in our study were not high enough to cause a long-term effect. Similar results of an initial transitory decrease in feed consumption was reported in a subacute study performed in our lab where mink were fed 6.25, 12.5, or 25 ppm ergot alkaloids for 30 days (Weinstein *et al.*, 1999)

Decrease in body weight has been reported to be a characteristic of ergot toxicity in animals, but there are studies that have indicated no significant effect on body weight. In a study where rats were fed ergometrine (2, 10, 50, or 250 ppm), the ergot alkaloid caused a numerical, but non-significant, decrease in body weight compared to controls (Peters-Volleberg *et al.*, 1996). Studies that reported a decrease in body weight gain in animals also reported a decrease in daily feed intake. Piper *et al.* (1997) reported that rats fed diets with 50% endophyte-infected fescue had decreased feed intake and decreased

average daily weight gain. In our study, consumption of ergot alkaloids had no significant effect on body weights of adult female mink. All mink lost a similar amount of weight during the 19-week trial. Since there was only a transitory decrease in feed consumption that was no longer apparent after 7 days, it is not surprising that body weights were not significantly affected.

One of the most important effects of ergot alkaloids is the decrease in plasma prolactin concentrations, as was observed in the present study. The control of prolactin secretion from lactotrophs of the anterior pituitary is primarily through inhibition of dopamine, which occurs in the hypothalamus or posterior pituitary. Dopamine is then transferred to the anterior pituitary via the hypothalamic/hypophysial portal system where it exerts its inhibitory action on prolactin secretion through its interaction with the D2 dopamine receptor located on the lactotrophic cell. Compounds that interact with this receptor as agonists, such as ergot alkaloids, are known to cause suppression of prolactin release (Cross *et al.*, 1995). Decreases in prolactin have been reported due to consumption of ergot alkaloids, including ergovaline, ergotamine, ergonovine, and ergometrine. In our study, ergotamine comprised 12.9% of the total ergot alkaloids present in the contaminated oats. The presence of ergotamine along with the other ergot alkaloids may have increased the circumstances of prolactin concentrations being reduced. This decrease in plasma prolactin concentration has been reported to influence different reproductive parameters.

Ergot alkaloids have been reported to affect the gestation length in some species of animals. Although the mechanism by which ergot alkaloids affect gestation has not been fully elucidated, some studies have shown a relationship between serum prolactin

concentrations and gestation length (Polejaeva *et al.*, 1997). Mink are seasonal breeders with late February through March being the breeding season. They are induced ovulators with the ability to ovulate a second wave of follicles by mating 8-10 days after the first ovulation (Polejaeva *et al.*, 1997). In induced ovulators such as mink, the embryos resulting from the first wave survive by entering an obligatory period of diapause and delayed implantation. The reactivation of the embryo occurs within the first week following the equinox and the blastocyst enters the uterus on days 5-6 after mating and then enters diapause. Although the normal length of diapause is 15 to 25 days, manipulation of the photoperiod can either shorten (by 5 days) or lengthen (up to 55 days) diapause. The authors stated that the mechanism that terminates diapause is still poorly understood, but prolactin is a major component of the luteotropic complex that terminates embryonic diapause in mink (Papke *et al.*, 1980). In the study by Ploejaeva *et al.* (1997), daily injection of prolactin beginning on day 7 after mating was followed by a rapid increase in the plasma concentration of progesterone that occurred 10 days earlier in prolactin-treated female mink compared to untreated female mink. Prolactin-treated females whelped on average 10 days earlier than untreated females. In our study, consumption of ergot alkaloids decreased prolactin concentrations at all doses and increased the gestation period (significant at 6 ppm) compared to the controls (60 vs 47 days). The normal gestation period of mink mated in early March is 58 days whereas for mink mated in mid-March is 47 days. Control mink that were on reproductive trials in the same animal room as used for the present study had gestation lengths that ranged from 48.8 days to 64.5 days (Crum *et al.*, 1993; Heaton *et al.*, 1995; Powell *et al.*, 1996). The results of the study suggest that the ergot alkaloid-induced increase in gestation was

caused by the decrease in plasma prolactin concentrations although the range of gestation lengths in the present study was within the range for control mink. Similar increases in gestation length have been reported by Cross (1997) in horses consuming endophyte-infected tall fescue. The gestation length increased in horses by 27 days compared to the controls due to consumption of endophyte-infected tall fescue.

An ergot alkaloid such as ergotoxine can upset the balance of hormones that are necessary for normal pregnancy (Shelesnyak, 1957). Progesterone is an extremely important hormone for the maintenance of pregnancy as it is needed for the provision of uterine secretions in preparation for the implantation of the embryo and embryo motility. (Cross, 1997). Alterations in progesterone can affect embryo motility and hinder maternal recognition of pregnancy. Studies on mares consuming endophyte-infected tall fescue reported reduced levels of serum progesterone, but the mechanism by which the concentration of progesterone is altered or reduced has not been elucidated. Although progesterone was not measured in our study, it could be one of the reasons for the reduced reproductive rates in mink fed diets containing 6 or 12 ppm ergot alkaloids.

Many animal studies have reported reproductive effects to be the biggest problem caused by consumption of ergot alkaloids. The more common reproductive effects include failure to implant, fetal resorption, and prenatal mortality. Animal studies have shown that ergot alkaloids can affect reproduction in more than one way. The decrease in serum prolactin concentration can be one reason for reproductive failure in animals caused by consumption of ergot alkaloids. When ergotoxine-induced pregnancy failures were seen in rats, administration of prolactin was successful in reversing the effects of ergotoxine (Shelesnyak, 1958). Administration of prolactin to ergotoxine-fed rats

increased the percentage of successful pregnancies from 75 to 88%. In our study, consumption of ergot alkaloids decreased the serum prolactin concentrations in female mink. The decrease in prolactin concentrations could have been responsible for reproductive failures in mink fed 6 and 12 ppm of ergot alkaloids.

Some natural alkaloids of ergot increase uterine motor activity. Raymond (1995) demonstrated that a small dose of ergotamine caused the uterine smooth muscle to contract. As the dose of ergotamine increased and the interval between contractions decreased, there was sustained contracture. Besides the uterine contractions, ergot alkaloids can cause an impairment of blood supply to the uterus and placenta, mainly as a consequence of ergot-induced vasoconstriction (Grauwiler and Schon, 1973). This impairment in blood supply can lead to embryonic death (Franklin and Brent, 1964). In the present study, ergotamine comprised 12.9% of the total ergot alkaloids present. This could explain why there were lower numbers of mink whelping in the groups fed 6 and 12 ppm of ergot alkaloids in the present study.

Besides implantation failures in animals, ergot alkaloids have been reported to cause reduced litter size, increase prenatal and neonatal mortality, and congenital deformities. Grauwiler and Schon (1973) reported increased prenatal mortality in rats, mice, and rabbits fed ergotamine (5 mg/kg diet for rats, 25 mg/kg diet for mice, and 1 mg/kg diet for rabbits). The authors came to the conclusion that the effects on the offspring were largely due to an indirect disturbance of fetal development, secondary to impairment of normal pregnancy by subchronic doses of ergotamine. In our study, the groups fed 6 and 12 ppm ergot alkaloids (consisting of 12.9 % ergotamine) had reduced litter sizes, and evidence of congenital deformities. These effects could be attributed to

the vasoconstrictive effects of ergot alkaloids. Evidence of increased incidence of fetal resoprtions have been reported by Carpent and Desclin (1969) when rats were given 1 mg/kg body weight of ergocornine on day 7 of gestation. In the same study, ergocornine given at a later stage of gestation had no effect on pregnancy. The authors explained that the fetal resorption was not caused directly by ergocornine, but indirectly by ergocornine-induced progesterone deficiency. This may explain the fetal resorption seen in females fed 12 ppm ergot alkaloid in our study, although progesterone was not determined.

It has been reported that an average of 12 percent mortality of kits from birth to 1 week and 7 to 24 percent mortality from birth to 3 weeks of age is normal in mink (Howell, 1979; Hozbor *et al.,* 1996). In our study, the group fed 3 ppm ergot alkaloid had 17 percent mortality at 3 weeks from birth, but in the 6 ppm ergot alkaloid group, there was 100 percent mortality. The increase in mortality may be due to consumption of ergot alkaloids because in the control group, there was only 15 percent mortality, which is within the normal values.

Another effect of ergot alkaloids that has been reported in many reproductive studies is the reduced body weight of the young from females fed diets containing ergot alkaloids. The reasons for reduced body weight of the young have been attributed to failure to lactate because of impaired maternal instinct (Orth and Ritchie, 1947), reduced maternal feed intake (Tindal, 1956), inhibition of the milk ejection reflex (Grosvener, 1956), and failure of the young to nurse (Sommer and Buchanan, 1955). Sommer and Buchanan (1955) stated that pups from ergot alkaloid-fed rats could not suckle milk as vigorously as pups from the control groups, and that females in the treated groups may not have had enough milk for the pups. Also, animals like sheep, mice, and cattle have

shown reduced milk yields, whereas horses have shown complete agalactia due to consumption of endophyte-infected fescue grass (Cross, 1997). Although we did not examine the mammary glands, our study indicated that kits born to females consuming high doses of ergot alkaloids were not well nourished. This was particularly apparent when the kits were 3 weeks old because the kits from the control group were 43 % heavier compared to kits of females fed 3.0 ppm ergot alkaloids. Normal body weights for 3-week-old kits range from 100 to 147 g (Travis and Schiable, 1961; Tauson, 1994). Since the kits were receiving nourishment only from the mother during the first 3 weeks of their life, a decrease in milk production would have a significant effect on kit body weight. Lactation could have been decreased due to the inhibitory effects of ergot alkaloids on prolactin because the promotion of mammary glands and initiation and maintenance of lactation are the principal functions of prolactin (Cowie and Tindal, 1971).

Besides prolactin, the involvement of progesterone and estrogen in lactation is significant (Cross *et al.*, 1995). Estrogen and progesterone stimulate the development of ductal and secretory structures when mammary tissue is primed with insulin, aldosterone and prolactin. Estrogen is necessary for the cell division in terminal end buds that leads to ductal growth whereas progesterone stimulates lobulo-alveolar growth. Prolactin is necessary to prime mammary tissue and apparently acts synergistically with estrogen and progesterone to promote mammary tissue growth. Cross and associates (1995) explained that progesterone and prolactin levels were lower in mares consuming endophyte-infected fescue, but estradiol-17β concentration were higher compared to mares on the control

diet. Although progesterone and estrogen was not measured in our study, alterations in these hormones could have contributed to the decrease in lactation.

The effect of ergot alkaloids on blood chemistry parameters has not been studied extensively, but there are a few studies that have reported variable effects. Changes in all blood chemistry analytes do not occur simultaneously, and some animals may require extended periods of consumption of endophyte-infected fescue to cause alterations in serum biochemistry profiles (Schultze *et al.*, 1999). This may be why we did not see a consistent effect of ergot alkaloids on all blood chemistry parameters.

Consumption of ergometrine has been reported to cause a decrease in plasma glucose concentration in rats (Peters-Volleberg *et al.*, 1996). The authors explained that ergometrine affects carbohydrate metabolism, but the mechanism of action is not known. In the present study, the serum concentration of glucose was significantly higher prior to breeding and significantly lower post-whelping in the females fed 12 ppm ergot alkaloids. Ergotmetrine was not one of the alkaloids present in the contaminated oats used in this study, but it is possible that the other ergot alkaloids may have caused a decrease in the plasma glucose concentration.

Increased activity of creatnine kinase in serum indicates damaged muscle (Coles, 1986). The creatnine kinase activity of mink fed 12 ppm ergot alkaloid was considerably higher (1859 IU/l) compared to the control value (559 IU/l). Normal creatnine kinase activities for mink are in the range of 705 IU/l (Restum *et al.*, 1995). It is not clear why the creatnine kinase activity was elevated that high.

Alanine aminotransferase is an enzyme within the cytoplasm of hepatic parenchymal cells that catalyzes the transamination of l-alanine and 2-oxoglutarate to

pyruvate and glutamate (Schultze *et al.*, 1999). Increased alanine aminotransferase activity occurs due to a leakage of the enzyme from injured hepatocytes. In our study, ergot alkaloids caused a significant decrease in alanine aminotransferase concentration, and thus the biological significance of this change is unknown.

Hyperkalemia (increase in serum potassium concentration), and hyperphosphatemia (increase in serum phosphorus concentration) indicate acute renal failure (Coles, 1986). In the present study, the post-whelping concentrations of potassium and phosphorus were significantly higher in the 12 ppm ergot alkaloid group compared to control values (5.48 vs 4.40 mmol/l and 6.49 vs 4.08 mmol/l, respectively). Reported normal values for potassium range from 4.1 to 5.0 mmol/l and the reported normal value for phosphorus is 5.9 mmol/l (Blumenkrantz and Blomstedt, 1987; Wamberg, 1992). Histological assessment indicated no renal damage. This may mean that even though the values are statistically significant, they are not biologically significant because they are relatively close to normal values reported for mink.

In general, although changes in serum chemistry parameters due to consumption of ergot alkaloids have been reported in different animal species, the mechanisms of action are usually unknown. Schultze *et al.* (1999) stated that explanations and /or speculation for these phenomena include effects secondary to reduced feed intake, deficiency of vitamins, proteins, or minerals, development of inhibitors of enzymic action, decreased production due to decreased cell proliferation, increased clearance of enzymes via liver or kidneys, and alterations in conditions like pH and tonicity that may inhibit enzymic action.

Studies that have focused on the histopathological changes in animals induced by consumption of ergot alkaloids have reported changes occurring only in the liver. Rats fed ergometrine had relatively large and occasionally swollen hepatocytes (Peters-Volleberg *et al.*, 1996). This kind of hepatocellular appearance is considered typical of excessive glycogen storage, and it was seen only in rats fed 250 mg ergometrine/kg diet. The authors explain that the glycogen content of most cells appeared to be relatively low, which was considered to be due to the artificial loss of glycogen in liver samples during storage in formalin or during histological processing. Thus, no evidence of ergometrine-induced hepatocellular degeneration or necrosis or other liver abnormalities were reported. In our study, consumption of ergot alkaloids had no effect on tissue histopathology. The only explanation for this may be that the concentration of ergot alkaloids used in our study may not have been high enough to cause any tissue damages.

It is difficult to compare clinical signs seen in our mink study with studies utilizing other species. In our study, we used a mixture of ergot alkaloids in the diet whereas previous studies on other animals have used either individual ergot alkaloids or a mixture of 1 or 2 alkaloids.

Based on the results of our study, several things can be taken into consideration for future studies. Unlike our study where feed consumption was measured for the first 7 days of the trial, it could be measured for a longer period of time. One of the reasons for decrease in feed consumption in other animals was due to heat stress. For future studies, feed consumption can be measured at a time when the temperatures are warmer to see if heat stress actually decreases feed consumption. Also, the concentrations of ergot

alkaloids could be increased to see if higher levels of ergot alkaloids decrease feed consumption.

The decrease of prolactin concentration has been a major problem due to consumption of ergot alkaloids and this decrease in turn was related to other reproductive problems such as increase in gestation length and reproductive failures. In future studies, supplemental prolactin could be administered to females fed diets containing ergot alkaloids to determine if the supplemental prolactin could decrease gestation length or alleviate other reproductive problems.

As discussed earlier, progesterone is responsible for the maintenance of pregnancy because it is needed for the provision of uterine secretions in preparation for the implantation of the embryo and its utility. Although progesterone was not measured in our study, future studies could be designed to measure progesterone concentrations to see if the decrease in progesterone concentration is related to any reproductive problems in mink.

In our study, there was 100 percent mortality at 3 weeks from birth in kits whelped by females fed 6 ppm ergot alkaloids. One of the reasons for this mortality was attributed to a decrease in lactation, but we did not examine the mammary glands in females. Mammary glands could be examined in future studies to determine if consumption of ergot alkaloids causes a decrease in lactation.

Another thing that could be considered for future studies is to perform a long-term study (the present study was a 133 day trial). In our study, the kits were euthanized when they were 3 weeks old. Instead, they could be continued on the ergot alkaloid diet through the next generation to see how that may affect reproduction. Alternatively, the

kits could be fed the ergot alkaloid diet for at least 6 months instead of 3 weeks to determine how that may affect their growth and survivability. Our study did not show any histopathological changes in any tissues in the ergot-treated adult mink. Histopathological changes could be determined in kits fed diets containing ergot alkaloids from birth to 6 months of age.

In our study, we used a mixture of ergot alkaloids in different proportions in the diet fed to mink. We do not know which of these alkaloids caused which effects. In future studies, we could use individual alkaloids to study their effects.

Finally, higher doses of ergot alkaloids could be used for a long-term study in mink. Although a previous study in our laboratory (Weinstein *et al.*, 1999) used higher doses of ergot alkaloids, it was a 30-day trial and hence no effects were seen. Similar doses as Weinstien's could be used for a longer period of time.

Conclusion

Dietary concentrations of 6 or 12 ppm ergot alkaloids caused a transitory decrease in feed consumption of adult female mink over the first 7 days of the trial. The consumption of 6 ppm or higher ergot alkaloids caused a decrease in the number of females whelping compared to the controls. Ergot alkaloids also increased the gestation length in mink at 6 ppm or higher. Although the gestation length of the single mink in the 12 ppm group was only 62 days, there were mink in the 3 and 6 ppm ergot alkaloid groups that did not whelp but still had viable kits at as long as 78 days of gestation.

Kit mortality and survivability were also affected by the consumption of ergot alkaloids. At doses of 6 ppm or higher, there was 100% mortality of kits before 3 weeks

of age. Consumption of ergot alkaloids caused kit body weight in the 3 ppm ergot alkaloid group to be lower compared to the control kit body weight.

The post-whelping concentration of plasma prolactin decreased significantly at doses of 3 ppm or higher of ergot alkaloids. Since prolactin is responsible for mammary gland promotion, and initiation and maintenance of lactation, the decrease in serum prolactin concentration may have had an effect on kit mortality as the only nourishment the kits were receiving for the first 3 weeks after birth was their mother's milk.

The results of the study indicated that consumption of 3 ppm or higher doses of ergot alkaloids have an adverse effect on plasma prolactin concentration, litter size, kit birth weight, and kit body weight at 3 weeks of age. The study also indicated that consumption of 6 ppm or higher doses of ergot alkaloids had adverse effects on gestation length, the number of females whelping, and kit survivability at 3 or more weeks of age. Thus, consumption of ergot alkaloids at doses of 3 ppm or more caused reproductive toxicity in adult female mink.

Because our study concluded that ergot alkaloids have an adverse effect in mink, mink farmers should avoid using ergot-contaminated grains and grasses in the mink diet. Mink fur is one of the most expensive and precious components of the fur industry, especially used in making fashionable coats and purses. Avoiding the use of ergot-contaminated feed components in mink diets can result in better production of fur, both in quantity and quality. Our results indicate that 3 ppm or higher concentration of ergot alkaloids had an adverse effect on mink reproduction and survivability. Since 3 ppm was the lowest concentration used in the present study, it would be beneficial to mink farmers if a no observable adverse effect level (NOAEL) could be identified.

References

Aulerich, R. J., S. J. Bursian, and A. C. Napolitano. 1987. Toxicity of mycotoxins to mink. Progress Report of Projects Supported by the Mink Farmers Research Foundation, Thiensville, WI. 5 pp.

Bennett, D. R., R. P. Rathmacher, and R. L. Cochrane. 1977. Effects of pseudopregnancy and ergocornine treatment on synchronization of mating, duration of gestation and production of young in rats. J. Anim. Sci. 46:768-775.

Bennett-Wimbush, K. and W. E. Loch. 1997. A preliminary study of the efficacy of fluphenazine as a treatment for fescue toxicosis in gravid pony mares. J. Equine. Vet. Sci. 18:169-174.

Blumenkrantz, N. and L. Blonstedt. 1987. Chelators depigment and increase elasticity of mink skin. Acta Agric. Scand. 37:375-395.

Boissier, J. R. 1978. General pharmacology of ergot alkaloids. In: *Ergot Alkaloids* (P. F. Spano and M. Trabucchi, Eds.) S. Karger AG, Switzerland. pp. 12-26.

Bolt, D. J., J. Bond., G. P. Lynch, and T. Elsasser. 1982. Concentrations of PRL, LH, FSH, GH, and TSH in plasma and pituitary of ewes grazing tall fescue and orchardgrass pastures. J. Anim. Sci. 55:4.

Browning, R., Jr., F. N. Schrick., F. N. Thompson, and T. Wakefield., Jr. 1998. Reproductive hormonal responses to ergotamine and ergonovine in cows during the luteal phase of the estrous cycle. J. Anim. Sci. 76:1448-1454.

Browning, R., Jr., F. N. Thompson., J. L. Sartin, and M. L. Leite-Browning. 1997. Plasma concentrations of prolactin, growth hormone, and luteinizing hormone in steers administered ergotamine or ergonovine. J. Anim. Sci. 75:796-802.

Browning, R., Jr., M. L. Leite-Browning., H. M. Smith, and T. Wakefield., Jr. 1998. Effect of ergotamine and ergonovine on plasma concentrations of thyroid hormones and cortisol in cattle. J. Anim. Sci. 76:1644-1650.

Bursian, S. J., R. J. Aulerich., J. K. Cameron., N. K. Ames, and B. A. Steficek. 1992. Efficacy of hydrated sodium calcium aluminosilicate in reducing the toxicity of dietary zearalenone to mink. J. Appl. Toxicol. 12:85-90.

Carpent, G., and L. Desclin. 1969. Effect of ergocornine on the mechanism of gestation and on fetal morphology in the rat. Endocrinology. 84:315-324.

Cheeke, P. R. and L. R. Shull. 1985. *Natural Toxicants in Feeds and Poisonous Plants*. AVI Publ. Co., Westport, Conneticut. 492 pp.

Christensen, C. M. 1980. Ergots. In *Mycotoxins and Animal Feeds*. U. S. Department of Commerce, Springfield, Virginia. pp. 1-44.

Coles, E. H. 1986. *Veterinary Clinical Pathology*. W. B. Saunders Company. Philadelphia, PA. 486 pp.

Cowie, A. T, and J. S. Tindal. 1971. The physiology of lactation. In: *Monographs of the Physiological Society*. (H. Davson and R. Whittorn, Eds) London.

Cross, D. L. 1997. Fescue toxicosis in horse. In *Neotyphodium/Grass Interactions* (C. W. Bacon and N. S. Hill, Eds.). Plenum Press, New York. pp 289-309.

Cross, D. L., L. M. Redmond, and J. R. Strickland. 1995. Equine fescue toxicosis: signs and solutions. J. Anim. Sci. 73:899-908.

Crum, J. A., S. J. Bursian, R. J. Aulerich., P. Polin, and W. E. Braselton. 1993. The reproductive effects of dietary heptachlor in mink (*Mustela vison*). Arch. Environ. Contam. Toxicol. 24:156-164.

Flieger, M., M. Wurst, and R. Shelby. 1996. Ergot alkaloids - sources, structures, and analytical methods. Folia Microbiol. 42:3-30.

Fluckiger, E., M. Marko., W. Doepfner, and W. Niederer. 1976. Effects of ergot alkaloids on the hypothalamic pituitary axis. J. Postgrad. Med. 52:57-61.

Franklin, J. B. and R. L. Brent. 1964. The effect of uterine vascular clamping on the development of rat embryos three to fourteen days old. J. Morph. 115:273-290.

Genter, M. B., W. M. Hagler., J. A. Hansen., B. A. Mowrey., F. T. Jones., M. H. Poore, and L. W. Whitlow. 1999. Mycotoxins and animal health. North Carolina State University Cooperative Extension. 2 pp.

Grauwiler, J. and Schon, H. 1973. Teratological experiments with ergotamine in mice, rats, and rabbits. Teratology. 7:227-236.

Griffith, R. W., J. Grauwiler., C. Hodel., K. H. Leist, and B. Matter. 1978. Toxicologic considerations. In *Handbook of Experimental Pharmacology* (B. Berde and H. O. Schild, Eds) Springer-Verlag, Germany. 49: 805-851.

Grosvenor, C. E. 1956. Effect of ergotamine on milk-ejection in lactating rat. Proc. Soc. Exp. Biol. Med. 91:294-296.

Heaton, S. N., S. J. Bursian, J. P. Giesy., D. E. Tillitt., J. A. Render., P. D. Jones., P. A. Verbrugge., T. J. Kubiak, and R. J. Aulerich. 1995. Dietary exposure of mink to carp

from Saginaw Bay, Michigan. 1.Effects on reproduction and survival, and the potential risks to wild mink populations. Arch. Environ. Contam. Toxicol. 28:334-343.

Hofmann, A. 1978. Historical view on ergot alkaloids. In *Ergot Alkaloids* (P.F. Spano and M. Trabucchi, Eds.). S. Karger AG, Switzerland. pp.1-11.

Howell, R. E. 1979. Causes of early kit losses in finely-bred dark mink studied. Fur Rancher. 54:8,13.

Hozbor, F. A., H. Crenovich, and M. P. Lomonaco. 1996. Death occurrences in mink farms (*Mustela vison*) in Argentina. Scientifur. 20:299-303.

Jackson, J. A., S. G. Yates., R. G. Powell., R. W. Hemken., L. P. Bush., A. Boling., P. M. Zavos, and M. R. Seigel. 1989. Physiological responses in rats fed extracts of endophyte-free and endophyte-infected tall fescue seed relative to some known ergot alkaloids. Drug and Chem. Tox. 12:147-164.

McQueen, R. D. 1993. Effect of ergot toxins on cattle. Herd and Animal Health, Univ. Illinois, Urbana (October, 1993). 3 pp.

Orth, O. S and G. Ritchie. 1947. A pharmacological evaluation of dihydroergotamine methanesulfonate. J. Pharmacol. Exp. Therap. 90:166-173.

Papke, R. L., P. Concannon., H. F. Travis, and W. Hansel. 1980. Control of luteal function and implantation in the mink by prolactin. J. Anim. Sci. 50:1102-1107.

Paterson, J., C. Forcherio., B. Larson., M. Samford, and M. Kerley. 1995. The effects of fescue toxicosis on beef cattle productivity. J. Anim. Sci. 73:889-898.

Peters-Volleberg, G. W. M., R. B. Beems, and G. J. A. Speijers. 1996. Subacute toxicity of ergometrine in rats. Food and Chem. Toxicol. 34:951-958.

Piper, E. L., M. S. Gadberry., T. M. Denard., Z. Johnson, and M. Fleiger.1997. Effect of feeding ergovaline and ergine on growing rats. In *Neotyphodium/Grass Interactions* (C. W. Bacon and N. S. Hill Eds.). Plenum Press, New York. pp 437-439.

Polejaeva, I. A., W. A. Reed., T. D. Bunch., L. C. Ellis, and K. L. White. 1997. Prolactin-induced termination of obligate diapause of mink (*Mustela vison*) blastocysts *in vitro* and subsequent establishment of embryonic stem-like cells. J. Rep. Fert. 109:229-236.

Porter, J. K, and F. N. Thompson. 1992. Effects of fescue toxicosis on reproduction in livestock. J. Anim. Sci. 70:1594-1603.

Powell, D. C., S. J. Bursian., C. R. Bush., J. A. Render., G. E. Rottinghaus, and R. J. Aulerich. 1996. Effects of dietary exposure to fumonisins from *Fusarium moniliforme*

culture material (M-1325) on the reproductive performance of female mink. Arch Environ. Contam. Toxicol. 31:286-292.

Rall, T. W. and Schleifer, L. S. 1980. Oxytocin, prostaglandins, ergot alkaloids, and other agents. In *The Pharmacological Basis of Therapeutics*. (Goodman and Gillman, Eds.) New York, NY. pp 935-950.

Raymond, G. V. 1995. Teratogen update: ergot and ergotamine. Teratology. 51:344-347.

Restum, J. C., S. J. Bursian, M. Millerick, J. A. Render, A. J. Merrill Jr., E. Wang, G. E. Rottinghau, and R. J. Aulerich. 1995. Chronic toxicity of fumonisins from *Fusarium moniliforme* culture material (M-1325) to mink. Arch. Environ. Contam. Toxicol. 29:545-550.

Rutschmann, J. and P. A. Stadler. 1978. Chemical Background. In *Ergot Alkaloids and Related Compounds, Handbook of Expt. Pharmacology*. (B. Berde and H. O. Schild, Eds.) Berlin, NY. pp 29-78.

Samford-Grigsby, M. D., B. T. Larson., J. C. Forcherio., D. M. Lucas., J. A. Paterson, and M. S. Kerley. 1997. Injection of a dopamine antagonist into holstein steers to relieve symptoms of fescue toxicosis. J. Anim. Sci. 75:1026-1031.

Schmidt, S. P., D. A. Danilson, J. A. Holliman, H. W. Grimes, and W. B. Wegbster. 1986. Fescue fungus suppresses growth and reproduction in replacement beef heifers. Highlights of Agric. Res. 33:15. Alabama Agric. Exp. Sta., Auburn Univ., Auburn.

Schultze, A. E., B. W. Rohrbach., H. A. Fribourg., J. C. Waller, and J. W. Oliver. 1999. Alterations in bovine serum biochemistry profiles associated with prolonged consumption of endophyte-infected tall fescue. Vet. Human Toxicol. 41:133-139.

Shaar, C. J. and J. A. Clemens. 1972. Inhibition of lactation and prolactin secretion in rats by ergot alkaloids. Endocrinology. 90:285-288.

Shelesnyak, M. C. 1954. Ergotoxine inhibition of deciduoma formation and its reversal by progesterone. Amer. J. Physiol. 179:301-304.

Shelesnyak, M. C. 1955. Disturbance of hormone balance in the female rat by a single injection of ergotoxine ethanosulphonate. Amer. J. Physiol. 180:47-49.

Shelesnyak, M. C. 1957. Gonadotrophin content of pituitary of pregnant and pseudopregnant rats following single injection of ergotoxine. Endocrinology. 60:802-803.

Shelesnyak, M. C. 1958. Maintenance of gestation in ergotoxine-treated pregnant rats by exogenous prolactin. Acta Endocrin. 27:99-109.

Silberstein, S. D. 1997. The pharmacology of ergotamine and dihydroergotamine. Headache. 37:S15-S25.

Sommer A. F. and A. R. Buchanan. 1955. Effects of ergot alkaloids on pregnancy and lactation in the albino rat. Amer. J. Physiol. 180:296-300.

Tauson, A. H. 1994. Postnatal development in mink kits. Acta Agric. Scand. (Sect. A). 44:177-184.

Tindal, J. S. 1956. The effect of ergotamine and dihydroergotamine on lactation in the rat. J. Endocrin. 14:268-274.

Travis, H. F. and P. J. Schaible. 1961. *Fundamentals of Mink Ranching*. Circ. Bull. 229. Coop. Ext. Serv., Mich. St. Univ., E. Lansinf, MI. 101 pp.

Wamberg, S., T. N. Clausen, C. R. Olesen, and O. Hansen. 1992. Nursing sickness in lactating mink (*Mustela vison*). II. Pathophysiology and changes in body fluid composition. Canad. J. Vet. Res. 56:95-101.

Weinstein, P., R. J. Aulerich, and S. J. Bursian. 1999. Toxicity of ergot to mink. Progress Report to the Mink Farmers Research Foundation, Corvallis, OR. 8 pp.

Woloshuk, C. P. Mycotoxins and mycotoxin test kits. (Online) Available http://www.btny.purdue.edu/extension/pathology/BP/BP47/, 1/9/1997.