REPRODUCTION AND ENZYME DETOXIFICATION ACTIVITIES IN MOUSE LINES SELECTED FOR RESPONSE TO FESCUE TOXICOSIS

by

Catherine Ann Robertson Wagner

Thesis submitted to the Faculty of the

Virginia Polytechnic Institute and State University

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

in

Animal and Poultry Sciences
(Animal Breeding and Genetics)

APPROVED:

W. D. Hohenboken, Chairman

D. J. Blodgett

E. A. Wong

April, 1999
Blacksburg, VA

Key Words: *Festuca arundinacea*, Mice, Reproductive Performance, Glutathione Transferase, UDP

REPRODUCTION AND ENZYME DETOXIFICATION ACTIVITIES IN MOUSE LINES SELECTED FOR RESPONSE TO FESCUE TOXICOSIS

by

Catherine Ann Robertson Wagner
William D. Hohenboken, Chairman

Department of Animal and Poultry Sciences

(ABSTRACT)

In previous work, mouse lines were selected for resistance (R) or for susceptibility (S) to fescue toxicosis based upon reductions in post-weaning growth rate caused by an endophyte-infected diet. The first objective of the current experiment was to determine whether long term reproduction of S mice was more severely depressed than that of R mice by the toxic diet. The second objective was to quantify glutathione-S-epoxytransferase (GST) and uridine diphosphate glucuronosyl-transferase (UDPGT) activities in R and S dams form the experiment and to determine whether reproduction during continuous cohabitation and liver detoxification enzyme activities were correlated within line x diet groups. Effects of the toxic diet were detectable within the first litters produced. Reproduction was more seriously influenced by the toxic diet within the S line than within the R line when these measures were compared within four equal time phases. The effects of the toxic diet on reproduction were greatest early in the experiment; by the fourth time phase differences among line x diet groups, with the exception of litter weight, were not significant. Percentage differences in total reproduction were greater between S

mice fed the non-toxic diet and S mice fed the toxic diet than those between the R mice fed the non-toxic and toxic diets. Averaged across diets, GST activities were higher in R mice, but UDPGT activities were not significant. Within R line mice, GST was correlated with three reproductive measures, but UDPGT activity was not correlated with reproduction within any line x diet group.

Acknowledgments

I have been in Blacksburg seven years now. When I look back, I can't believe how much my life has changed and how much I've learned from the good times and especially from the bad. I owe a huge debt to the people who have helped me make this place my home.

I appreciate Dr. Blodgett's crash courses in grammar and toxicology, and hope that I didn't massacre either too badly.

Dr. Hohenboken showed me the magic behind science. He taught me to question what I'm told and not to accept without thought. I learned something about the practical side of life, too. I know now that you can go four-wheeling in a fifteen passenger van. I understand the mystery behind why one side of the "V" of geese in flight is longer than the other. I can truly appreciate good barbecue.

Dr. Lawrence taught me that there are several ways to work with horses and to be open-minded when observing them. Most of all, though, I learned that patience with horses and taking the time to try to understand their perspective teaches both of us the most.

Dr. Siegel accepts the hardships of life elegantly. He wants to discover everything he can about the world around him. I want to learn to do both these things.

Dr. Wong has endless patience and an expansive sense of humor. Fortunately.

Ok, Dr. Wood, pigs aren't that bad.

I spent a lot of time in the basement of Litton Reaves. Thanks, Diana, for making it pretty enjoyable. Give Homer II a hug for me.

I definitely owe Linda Correll, and I promise I'll never forget a plate blank again.

Bent Tree Farm is my refuge. I will truly miss the people there who have taught me so much about Saddlebreds and about working hard. I'll see you all at Louisville.

Mandy, Martha, what can I say? Though you probably got me into a lot of trouble, you also got me out of some. I don't think I've ever had as much fun (or spent as much money) as when I've been out with you all. There are friends of the heart and friends of the road, and I know we'll always be a part of each other's lives.

My parents have wholeheartedly supported me emotionally (and

financially) when times were tough. They eventually got used to the animal stories at the dinner table and learned how to identify Black Baldies driving down the interstate. They only occasionally ask “When are you going to get a real job?” and I’d like to think they are just kidding.

Table of Contents

List of Tables

page

List of Figures

page

Introduction

Previous work leading to mouse line development

This thesis describes two projects that are components of a body of work established by Dr. Bill Hohenboken to examine genetic variation in response to toxic fescue challenge. Initially, studies examined genetic variation within cattle populations. In 1991, Hohenboken et al. examined an Angus cow population for responses to fescue seed in the diet. Cows were ranked based upon serum prolactin levels, serum cholesterol, and body weight changes when they were challenged with toxic fescue seed in the diet. These criteria did not unambiguously identify susceptible cows and changes in serum prolactin were not clearly related to past reproductive performance.

In a subsequent 1993 study, Gould and Hohenboken examined the progeny of two Polled Hereford bulls. One of these sires, bred in Missouri, had a reputation for producing calves resistant to fescue toxicosis. On the toxic diet, calves of both sires suffered similar reductions in feed intake, prolactin concentration, and cholesterol levels. Alkaline phosphatase levels in progeny of the reputedly resistant sire were not reduced as much as levels in progeny of the control sire. The resistant reputation of the Missouri bull may have been earned, however. This bull's progeny maintained a lower core body temperature, which might have mitigated the effects of fescue toxicosis.

In an attempt to identify a laboratory animal model, Miller et al. (1994) compared the toxic challenge responses of several strains of inbred mice. The ADG of male mice fed a toxic diet was decreased in six lines but increased in a seventh. Toxic diets did not differentially affect line crosses between mouse strains identified as susceptible and resistant. A third experiment was able to identify a mouse strain that suffered less ADG reduction than another inbred line when both were fed toxic diets. An additional experiment showed that the mates of males from one line produced three fewer pups per litter than the mates of males from another line when these parents were placed upon a toxic diet. Miller et al. observed the litter size reduction in an additional experiment within the study but were not again able to confirm ADG differences. They concluded that concentrations of toxin and the life cycle stage at which a challenge is imposed may significantly alter the animal's response.

Instead of examining existing mouse lines for resistance or susceptibility, Hohenboken and Blodgett (1997) began an experiment to identify and select individuals within an outbred mouse population. Using reductions in post-weaning growth rate while under toxic challenge as a selection criterion, they were able to create two divergently selected mouse lines in eight generations. In addition, they examined liver detoxification enzyme activities within these lines in an attempt to pinpoint physiological coping mechanisms. Liver cytochromes p450 and b5 activities did not differ between lines, but glutathione-S-epoxytransferase (GST) and uridine diphosphate glucuronosyltransferase

(UDPGT) activities did. Resistant mice had higher GST and UDPGT activities than susceptible mice, even when not under toxic challenge.

Detoxification enzymology

Enzymes that detoxify foreign compounds (xenobiotics) within the body are divided into two general categories: phase I and phase II. Phase I enzymes, usually located within the endoplasmic reticulum, are responsible for functionalization of xenobiotics. These enzymes, such as the cytochrome p450 family, add or expose functional groups on xenobiotics to prepare the compound for metabolism by phase II enzymes. Uridine diphospho(UDP)glucuronosyltransferase and glutathione-S-transferase are the two most common phase II enzymes. Several factors, including nutrition, disease, hormonal status, age, sex, time of day or year, and species, may affect the rate of biotransformation.

During glucuronidation, UDP-glucuronosyltransferase conjugates UDP-glucuronic acid with the exposed functional group, which may be hydroxyl, carboxyl, amino, or sulfhydryl, on the xenobiotic substrate. Unlike most phase II reactions, this occurs in the endoplasmic reticulum. Glucuronosyltransferase has low substrate specificity and is not restricted to the products of phase I reactions. The newly-formed conjugate may be excreted in the urine (<250 m.w. in rats) or in the bile (>350 m.w. in rats). In addition to

increasing excretability of the xenobiotic by increasing its water-solubility, glucuronosyltransferase can also shield receptors from attack by attaching a bulky moiety to the toxic compound.

Glutathione-S-transferases are cytosolic enzymes with several isoforms that have low substrate specificity. The substrate must only be hydrophobic, electrophilic, and react non-enzymatically with the enzyme. Glutathione-S-transferases detoxify xenobiotic compounds in three ways. First, they may bind glutathione to the substrate and form the urinary metabolite mercapturic acid. Second, glutathione-S-transferases alone may bind to the xenobiotic to render it inactive and are thus termed ligandins. Third, glutathione-S-transferases may form covalent bonds with xenobiotics and are themselves rendered inactive in a suicide reaction. Compounds conjugated by glutathione-S-transferases may be excreted in the urine, bile, or feces. For a more comprehensive review, see deBethizy and Hayes (1994) or Sipes and Gandolfi (1991).

Objectives

The first part of this work examined the effect of toxic and non-toxic diets on previously-selected resistant and susceptible lines while they were under a reproductive stress, continuous cohabitation. If resistant mice are truly able to cope with toxic

challenge, any reduction in reproduction (offspring born, offspring weaned, number of litters produced, litter weaning weight) should be less than in susceptible parents.

The second part of this project examined GST and UDPGT enzyme activity levels in the livers of dams and determined the correlation between those activities and reproduction. Though Hohenboken and Blodgett (1997) found that GST and UDPGT levels differed between R and S lines, a more specific analysis may show that higher detoxification enzyme activities correlate with increased reproductive ability.

Review of Literature

Introduction

Tall fescue (*Festuca arundinacea* Schreb.) covers approximately 15 million hectares in the transition zone of the United States, an area which extends from southern Illinois and Ohio to northern Mississippi and includes Arkansas, Kentucky, Tennessee, and West Virginia and parts of Missouri, Arkansas, Louisiana, Alabama, Georgia, Virginia, and the Carolinas (Thompson et al., 1993). Although tall fescue has myriad attributes as a forage, including a nutritional profile that is equal in quality to other grasses grown in this area (Bush and Buckner, 1973), livestock are sometimes unable to perform well while grazing tall fescue pastures. The symbiotic relationship between tall fescue and an endophytic fungus, *Neotyphodium coenophialum* ((Morgan-Jones and Gams) Glenn, Bacon, and Hanlin), results in the production of toxic compounds that affect livestock species in different ways. Cattle may suffer fescue foot, fat necrosis, or summer syndrome, whereas horses experience reproductive difficulties (Hoveland, 1993). Sheep are less well-studied but appear more tolerant than cattle of the effects of toxic fescue (Bond et al., 1988; Hannah et al., 1990; Strickland et al., 1993). In addition, tall fescue may adversely affect various wildlife species such as deer, rabbits, and mice (Hoveland, 1993). Hoveland (1993) estimated that 8.5 million cattle, mostly used in cow-calf operations, graze endophyte infected (E+) tall fescue. He approximated that, annually,

the cattle industry lost $354 million as a result of unborn calves and $255 million due to reduced weaning weights, creating a total $609 million detriment. Other estimates place the cost to the cattle industry closer to $800 million per year (Strickland et al., 1993).

Tall Fescue and Its Impact on Animal Production

Characterization of endophyte-infected fescue

Nomenclature and Description. The endophytic fungus infecting tall fescue was first discovered and named *Epichlöe typhina* by Bacon et al. (1977). The fungus was taxonomically reclassified and renamed *Acremonium coenophialum* by Morgan-Jones and Gams in 1982 and recently changed again to *Neotyphodium coenophialum* based upon rDNA analysis (Glenn et al., 1996). The relationship between grass and endophyte is symptomless (Bacon et al., 1986) and infected fescue cannot be distinguished macroscopically (Johnson et al., 1982). Infection can be detected by either microscopic examination of tissue with aniline blue stain (Bacon et al., 1977) or, more efficiently, enzyme-linked immunosorbent assay (ELISA) testing for *N. coenophialum* antigens (Johnson et al., 1982). The fungus is located intercellularly within the leaf sheath, stem, and seed coat of the plant but not within the root or leaf blade (Hinton and Bacon, 1985; White et al., 1993). The non-sporulating fungus is transmitted through grass seed production but is not found within the seed embryo itself. The endophyte obtains

nutrition from the plant but can survive during seed storage, though for less than a year, using internal reserves (Hinton and Bacon, 1985).

Toxic chemical components. The chemical constituents of E+ fescue that cause toxicosis are poorly characterized and understood, but production of these compounds probably results from the interaction between the fungus and plant (Hinton and Bacon, 1985); non-endophyte infected (E-) plants are not toxic to potential predators (Bacon et al., 1986). The compounds implicated in animal toxicosis are loline alkaloids and ergot alkaloids. In E+ fescue, approximately 50% of the ergot alkaloid levels are ergopeptines and half of that fraction is ergovaline (Bacon et al., 1986). Ergopeptines are structurally similar to serotonin, dopamine, epinephrine, and norepinephrine (Strickland et al., 1993). By adding commercial preparations of ergot alkaloids to rat diets, Jackson et al. (1987) showed that ergonovine maleate, ergocryptine, and ergotamine tartrate, individually, had no effect on ADG or prolactin levels. However, these ergot alkaloids in combination did lower serum prolactin levels, possibly due more to increased dosage rather than to chemical interaction. Larson et al. (1992) showed that rats fed an E+ diet (325 ppb ergovaline) and treated with a dopamine antagonist (DA) had a significantly higher thermal circulation index (a measure of peripheral circulation and heat dissipation) than rats given E+ diets and a placebo (P). The E+P rats had reduced D2 dopamine receptor densities compared to those on other treatments, possibly because of ergot alkaloid

activity. Rats fed the E+DA treatment had D2 dopamine receptor densities similar to E-DA rats, but neither group had receptor densities equal to E-P levels.

Loline alkaloids, especially N-formylloline, caused reduced feed intake, a common symptom of toxicosis, but did not reduce serum prolactin or otherwise impact rat performance (Jackson et al., 1996). Similarly, Zavos et al. (1988a) showed that a methanol extracted fraction, which elutes N-acetyl and N-formyl lolines, but only trace amounts of ergopeptides, significantly reduced feed intake, ADG, and sperm motility and count in male rats.

Additional fescue extracts were examined in an attempt to identify the toxic compound from a range of chemical possibilities. Jackson et al. (1987) determined that a water extract contained components that reduced feed intake and lowered ADG in rats. However, a methanol extract was responsible for decreasing serum prolactin, suggesting that several chemical components work in tandem to produce fescue toxicosis. In a 1989 study, Jackson et al. examined ethanol extracts from E+ fescue and E- fescue. The E- extracts did not produce toxicity in rats; E+ ethanol extracts did. A combination of these and additional unidentified compounds probably resulted in the toxicosis (Jackson et al., 1989).

In addition, toxic compounds within E+ fescue vary by season and are affected by environmental conditions. Ergovaline concentration remained constant in leaf sheaths, leaf blades and seed heads from March through mid-June, but amounts within seed heads

increased dramatically in late June, decreased in early July and August, and increased again in mid-August (Rottinghaus et al., 1991).

Chemical mechanisms of toxicity. Dopamine is responsible for modulating prolactin secretion by binding to D2 dopamine receptors on the lactotrophs within the anterior pituitary. Dopamine agonists, such as ergot alkaloids, mimic dopamine and also bind to these receptors, decreasing serum prolactin (sPRL). Prolactin levels influence lactation, feed intake, and temperature regulation (Garner et al., 1993; Strickland et al., 1993).

Benefits of endophyte-infected fescue

Tall fescue, a perennial grass, is easily established, tolerant of poor agronomic management, resistant to pests and drought, and has a wide range of adaptation and an extended grazing season (Stuedemann and Hoveland, 1988). Toxins produced by E+ fescue confer resistance to the plant against a wide range of insect species, including cutworms, mealy bugs, crickets, and leaf hoppers. In addition, the endophyte/plant relationship protects the grass against nematode predation on roots. Endophyte-infected fescue may also be resistant to some fungal diseases like rusts. The mechanisms that produce this resistance are poorly understood (Latch, 1993).

Detriments of endophyte-infected fescue

Wildlife. Tannenbaum et al. (1998) showed that wild mice housed in the laboratory suffered similarly impaired reproductive function whether eating E- or E+ seed, with males more greatly impacted than females. Their interpretation was that wild mice cannot obtain enough nutritive support for reproduction from fescue seeds alone, and the mice are not affected by toxins in the seed. However, laboratory rodent populations are consistently affected by E+ seed diets (Daniels et al., 1981; Varney et al., 1987; Zavos et al., 1990). In addition, Coley et al. (1995) demonstrated reduced shrew, rat, and vole population densities in E+ grass stands compared to E- stands. New Zealand rabbit does suffered muscular tremors, increased urination, and elevated rectal temperatures and respiration rates after oral dosing with an E+ plant extract. In addition, fetal resorption, increased stillbirths, and agalactia were more common within the E+ treated group compared to the control animals (Daniels et al., 1984).

Sheep. Ewes pastured on high endophyte-containing fescue before breeding took longer to conceive or had a reduced conception rate compared to ewes on grass with low or zero endophyte infection. However, the ewes did not suffer reduced feed intake or body weight gains or show typical signs of toxicosis during or after gestation. Lamb birth

weight, survival, and weight gain were not adversely influenced by the high endophyte diet (Bond et al., 1988). Hannah et al. (1990) noted a linear decrease in the digestibility of E+ hay fed to sheep as ergovaline levels increased. In the same study, ergovaline and ergotamine diets resulted in elevated rectal temperatures when lambs were placed under 34˚C heat stress conditions.

Horses. Horses most commonly suffer reproductive difficulties including agalactia, prolonged gestation, improper foal presentation during birth, weak or stillborn foals, dysmature foals, thickened placenta, retained placenta, and rebreeding problems associated with consumption of E+ fescue (Monroe et al., 1988; Redmond et al., 1994; Cross, 1997). When comparing horses and cattle on high and low endophyte pasture, Aiken et al. (1993) found that both species suffered a reduction in average daily gain on the high endophyte diets. However, neither species suffered increased rectal temperature on toxic fescue. The study also suggested that horse breed types may vary in degree of susceptibility to toxicosis; however, only 12 horses were compared.

Cattle. Toxic fescue consumption manifests as one of three main pathologic conditions in cattle: fescue foot, fat necrosis, or summer syndrome (also termed "summer slump"). Fescue foot occurs mostly during cooler weather (< 15˚C) and is characterized by rough hair coat, emaciation, limb swelling, especially of the hind limbs, a red line

circumventing the coronary band, development of necrotic tissue, and sloughing of tail and ear tips in extreme cases (Cornell and Garner, 1983). Fat necrosis, or lipomatosis, results in deposition of hard, necrotic mesenteric fat around the intestinal tract, leading to impaired digestive function and sometimes death (Wilkinson et al., 1983). Both conditions are treatable only by removal of the animals from E+ pastures; the physiological damage cannot be wholly reversed.

Summer syndrome is the most prevalent and well-studied condition caused by E+ fescue ingestion. Cattle suffer reduced feed intake and ADG (Porter et al., 1990; Fribourg et al., 1991; Paterson et al., 1995). The two clinical signs may be cause and effect, or reduced ADG may result from decreased intake in combination with other physiological effects of the toxins. Peters et al. (1992) observed differences in animal performance although cattle on E+ and E- treatments consumed equal amounts. In the same study, Peters et al. noted that, at similar consumption levels, cattle on E+ diets did not show reduced ADG compared to cattle on E- diets in June, but they did gain less in August. This could be due to the fluctuation in ergot alkaloid levels discussed previously or could be a result of increased environmental temperatures. Pederson et al. (1986) found that incremental increases in E+ consumption resulted in linear ADG decreases, whereas Fribourg et al. (1991) suggested that an E+/clover diet produced a curvilinear ADG response, with animals more affected at lower amounts of E+. Cattle can compensate for some of this reduced ADG once they reach the feedlot, but managed to replace only 67%

of the reduced gain compared to contemporaries fed a low endophyte diet (Lusby et al., 1990). In contrast, Smith et al. (1986) saw no differences in feedlot performance after 56 d between high and low endophyte groups.

Reproductive difficulties, including decreased conception rates, decreased calf birth weights, decreased circulating progesterone, and reduced signs of estrus are other representative clinical signs of fescue toxicosis. Danilson et al. (1986) found that the conception rate of virgin crossbred beef heifers decreased linearly with increasing amounts of infected fescue in the diet. Birth weights of calves born to heifers that consumed toxic fescue during the last part of gestation were 3.7 kg lower than those of counterparts eating a toxin-free fescue diet (Bolt and Bond, 1986). After synchronization, weanling heifers penned with bulls on high endophyte pastures exhibited less estrus activity than heifers on low endophyte pastures. There were significantly more weanling heifers with normal ovarian activity, characterized as those that were acyclic and became cyclic or those who remained cyclic, in the group that consumed low endophyte fescue. In addition, in weanling heifers with a corpus luteum, those heifers on the high endophyte diets had reduced circulating progesterone. Yearling heifers on E+ pastures did not differ from their E- counterparts in ovarian activity, estrus expression, or progesterone levels but did suffer reduced growth (Mahmood et al., 1994).

Temperature-related physiological accommodations in cattle are another manifestation of fescue toxicosis and may be associated with decreased serum prolactin

(Bolt et al., 1983), a common consequence of the dopaminergic effects of ergot alkaloids (Bolt et al., 1983; Lipham et al., 1989; Porter et al., 1990; Fribourg et al., 1991). Cattle on endophyte-infected pastures have been observed salivating excessively, seeking shade, increasing their rate of respiration (Thompson and Stuedemann, 1993), and grazing more during the evening hours (Bond et al., 1984). Cattle grazing behavior changes in accordance with alterations in environmental stress. Cattle grazing heavily-infected tall fescue spent more time lying or standing in the shade and less time grazing when average ambient temperatures equaled 29.4°C than at 26.7°C. Compared to cattle consuming fescue with lower infection levels, these steers had rough hair coats, increased heart and respiration rates, and excessive salivation (Bond et al., 1984). Dry matter intake, rectal temperatures, and respiration rates of cattle consuming less toxic and more toxic fescue cultivars did not differ at 10°C to 13°C or 21°C to 23°C, but did differ significantly at 34°C to 35°C in studies conducted in environmentally controlled chambers (Hemken et al., 1981).

Fescue toxicosis may also affect immunocompetence. Serum hemagglutination titers and white cell counts in rats challenged with sheep red blood cells (SRBCs) were lower for rats fed a toxic diet than rats fed a non-toxic one. However, these rats also consumed less feed per day and differences may have been associated with nutritional deficiencies (Dew et al., 1990). Additionally, compared to steers on lowly endophyte-infected fescue pasture, steers grazing highly-infected fescue pastures had reduced

antibody response after immunization with tetanus toxoid (Dawe et al., 1997). In contrast, cattle challenged with concanavalin and sheep red blood cells while on endophyte-infected pastures mounted similar or even an increased humoral immune response compared to cattle grazing endophyte-free pasture (Rice et al., 1997).

Laboratory research models: mice and rats. As research into the causes of fescue toxicosis became more detailed, the need to develop a smaller, less expensive animal model became apparent. Fortunately, rats and mice suffer similar responses to cattle when challenged with toxic fescue and provide a ready solution to research needs. Compared to control rats, females consuming E+ extract had reproductive problems, including agalactia, fetal resorption, failed conception/embryonic death, and post-partum pup fatalities (Daniels et al., 1981). Neal and Schmidt (1985) found that increasing the amounts of toxic compounds in rat diets resulted in decreases of both feed consumption and ADG. Rats consuming a 50% rodent food 50% E+ seed diet had reduced water intake compared to counterparts that did not receive toxin. Studying female rats fed 0%, 5%, 10%, 20%, and 40% E+ diets, Varney et al. (1987) showed that high amounts (40%) of endophyte-infected feed significantly impaired reproductive function. The rats lost weight, had irregular estrous cycles, had reduced uterine weight upon necropsy, and more often failed to become pregnant. Rats fed a 20% E+ ration had extended estrous cycles, but did not suffer the extreme changes of their 40% counterparts. Rats fed 5% and 10% E+ diets did

not differ from the 0% group. In a second study, Varney et al. (1988) established that ADG, litter production, number of pups per litter, and pup weights were reduced by a toxin-containing diet. However, the treatment groups were fed restricted diets of equal volume to show that components within the forage, and not decreased intake, were responsible for the detrimental effects on animal health.

Male rats are also affected by toxic fescue. By comparing males on restricted E+ and E- diets, Zavos et al. (1986) concluded that males on the E+ treatment had slowed testicular and epididymal development and impaired sperm production. These findings agree with a 1988a study undertaken by Zavos et al. to examine the effect of methanol extract of fescue seed on male rats.

Mouse studies produced results similar to rat trials. Mice fed 60% E+ fescue had lower pregnancy and fertilization rates, fewer ova, and fewer viable embryos than female mice consuming 60% E- fescue feed. In that study, Zavos et al. (1988b) concluded that the reduced reproductive potential of animals on E+ diets was a result of higher embryonic mortality. They also suggested that ergot alkaloids interfere with hormonal balances necessary for pregnancy maintenance. In a study where both the E+ and the E- treatment groups were fed restricted diets, E+ mice were unable to support the nutritional demands of their pups as illustrated by differences in growth curves and weaning weights. Dams fed E+ diets also cannibalized their pups more frequently (Zavos et al., 1988c). Interestingly, young mice whose dams were fed E+ fescue throughout pregnancy gained

much more slowly through weaning even when they were grafted immediately after birth to foster mothers on a non-toxic diet. Female offspring of these fostered pups produced smaller litters than the female offspring of mice that were produced and suckled by dams consuming an E- diet. Though male CD-1 mouse groups showed no difference in ADG when fed an E+ diet compared to an E- one, they did suffer decreased epididymal weight, testes weight, and sperm motility (Varney et al., 1991).

A continuous 80 d breeding scheme in CD-1 mice fed E+ and E- diets showed that pregnancy rates, post-partum intervals, numbers of pups born per litter, and pups born dead or cannibalized differed between toxic and non-toxic dietary treatments. However, the dams' post-partum weight, the total weight of pups per litter, and the mean pup weight did not differ. In addition, mice were given three opportunities to litter and the pregnancy rate between toxic and non-toxic dietary groups was not different until the third parturition. Pregnancy rate did not differ between mice consuming a 40% or 60% E+ diet (Zavos et al., 1987).

Though not commonly used as an animal model for fescue toxicosis, Japanese quail fed E+ seed suffered reduced egg fertility and hatchability compared to quail fed restricted and *ad libitum* E- diets. However, there was no difference in egg production or the hatchability of fertile eggs among treatments (Zavos et al., 1993).

Combating Fescue Toxicosis

Chemotherapeutics. Perhaps because the causative agents of fescue toxicosis are incompletely understood, many types of vitamins, minerals, anthelmintics, hormones, vaccinations, and feed additives have been used to attempt to alleviate fescue toxicosis in sheep, horses, and cattle (Schmidt and Osborn, 1993). The most successful treatments include dopamine antagonists or dopamine receptor antagonists. Domperidone, a D2 dopamine receptor antagonist, is used to combat reproductive disorders in mares consuming E+ fescue during the last months of gestation. When fed domperidone, mares pastured on infected grass produced no stillborn foals, had improved milk production, and carried foals to terms similar to mares on E- pasture. Sulpride, another receptor antagonist, gave similar but lesser results (Redmond et al., 1994).

Metoclopramide, a dopamine antagonist, increased serum prolactin levels in steers consuming an E+ diet. It also improved feed consumption of cattle on both E- and E+ diets (Lipham et al., 1989). Metoclopramide increased serum prolactin in sheep but did not improve heart rate, respiration, or body temperature (Rankins, 1996). Another dopamine antagonist, synthetically produced Ro 24-0409, increased serum prolactin levels in steers and increased feed intake levels, but not to levels of steers on the E- diet (Samford-Grigsby et al., 1997).

Many clinical signs of fescue toxicosis mimic those of gastrointestinal parasitism, prompting the study of anthelmintics as a treatment. Ivermectin increased weight gain in

steers pastured on E+ fescue, although gain varied widely among studies (Bransby, 1997). Phenothiazine gave beneficial but non-significant results when used to reduce dorsal pedal vein contractility *in vivo*. Thiabendazole also had little effect (Oliver et al., 1992). Other unsuccessful treatments included estradiol implants (Beconi et al., 1995), yohombine hydrochloride (Jernigan et al., 1986), and selenium (Schmidt and Osborn, 1993).

IgG antibody levels were stimulated and weight gain on toxic feed temporarily increased in mice given vaccinations of bovine serum albumin-ergotamine conjugates and cholera toxin subunit B/ergotamine conjugates. Vaccinations did not, however, influence serum cholesterol or prolactin concentrations but did decrease alkaline phosphatase activity (Rice et al., 1998). Rabbits challenged with E+ seed after immunization against ergot alkaloids had increased feed intake but non-differing ADG when compared to non-immunized rabbits under the same challenge (Filipov et al., 1997).

Management. Several techniques for managing cattle grazing infected pastures or for managing the pastures themselves work well under certain combinations and circumstances. Mixing stands of fescue with other forages such as orchardgrass or clover seemed to dilute the effects of the toxin in the diet, but this method cannot totally overcome toxic effects (McMurphy et al., 1990; Chestnut et al., 1991; Thompson et al., 1993). In addition, overstocking the pastures or clipping them reduced seed-head formation, where endophyte levels are concentrated (Bransby et al., 1988). Other

methods for combating toxicosis include replanting E+ pastures with E- seed and grazing cattle on E- pastures during the hottest summer weather (Paterson et al., 1995).

Ammoniating toxin-containing seeds prior to feeding decreased ergovaline and N-formyl loline levels. Rats fed ammoniated seed showed increased daily feed intake, weight gain, and feed efficiency compared to rats consuming non-ammoniated E+ seed, but this management technique did not restore these factors to E- levels (Simeone et al., 1998). Interestingly, rats fed E+ fescue seed that had been incubated with steer ruminal fluid had improved intake, gain, and feed conversion compared to rats consuming non-incubated E+ seed. Rats consuming incubated or non-incubated E- seed diets did not differ with respect to intake, gain, and conversion rates, suggesting that ruminal fluid detoxified the seed rather than simply improving digestibility (Westendorf et al., 1992). Perhaps identifying components or microbes in ruminal fluid that detoxify fescue could lead to methods of seed treatment or to genetic selection for increased amounts of those components.

Genetics. Genetic selection of both cattle and plant species may contribute to a method of reducing fescue toxicosis in the animal industry. Genetic variation in response to toxic fescue within a cattle population may be masked by use of chemotherapeutics or livestock management. However, public concerns about exposure to drug residues in food and about animal welfare may limit the use of drug treatments. Genetic selection for

resistance to fescue toxicosis, especially in combination with other treatment methods, may prove to be more consumer-friendly (Morris, 1998).

Several studies have compared the relative resistance of cattle breeds and individuals within breeds, as well as the feasibility of selection for reduced toxins in the plant. Brown et al. (1996) examined milk yield and quality in Angus, Brahman, and reciprocal cross cattle when they were challenged with E+ pasture. Milk yield and milk fat were reduced more in Angus than Brahman cattle grazing E+ pastures, but milk protein concentrations were reduced more in the Brahman. A second study by Brown et al. (1997) examined direct breed effects and maternal heterosis of Angus, Brahman, and reciprocal cross cows fed E+ pasture. Calving rate was significantly reduced in purebred Angus cows on E+ pastures compared to purebred Angus cows on common bermudagrass, but the calving rate of Brahman cows did not differ between pasture treatments. Crossbred cows performed better than either purebred contemporary group under challenge. Aiken and Brown (1994) also showed that Angus x Brahman crossbred cows performed better than purebreds of the same breeds when consuming toxic fescue. Maternal heterosis was actually larger on E+ fescue pasture. In addition, crossbred steers performed better than their purebred contemporaries.

No interactions between mouse line and diet were observed in a mouse study comparing a randomly selected control line with mice selected for high prolificacy; E+ fescue affected reproduction and lactation similarly in both lines. This suggests that

livestock under intense selection for commercially important traits might not be affected to any greater extent than grade animals when fed toxic fescue. Secondly, a range in animal reproductive responses within each line suggested genetic variation within currently existing populations (Godfrey et al., 1994). This variation in response to fescue toxicosis indicates that populations may successfully respond to selection for resistance to the condition.

Within breeds, differences in lifetime production of Angus cows kept on fescue pasture could not be linked to fescue toxicosis resistance (Hohenboken et al., 1991). However, experimental design difficulties, such as providing adequate and uniform toxic challenge or maintaining appropriate environmental temperature, may have masked individual differences. In another study, 250 Angus bulls were evaluated for rectal temperature responses when challenged with E+ fescue seed diets. The most extreme responders were then mated to randomly chosen Angus heifers and their progeny examined for response to heat stress and endophyte challenge. Rectal temperatures did not differ between groups until the progeny were challenged with 200 ppb ergovaline. Calves produced by the bull with the greatest rectal temperature response showed a greater rectal temperature response than calves produced by the bull with the least temperature response (Lipsey et al., 1992).

Other research has attempted to reduce the toxin found in endophyte-infected fescue plants to ameliorate fescue toxicosis in cattle. Adcock et al (1997) found that selfed

plant progeny (genetically identical) showed wide variability in toxin levels, which suggests an environmental component influencing toxin concentration. These researchers estimated that the heritability during the first generation for low ergot alkaloid concentration was .56 and for high concentration was .49.

A genetic approach to reducing fescue toxicosis in cattle is unavoidably slow and will require careful planning and extreme patience. The degree of toxicity is determined by plant, endophyte, and animal genotypes, as well as their interactions. However, genetic improvement could provide long term and relatively permanent results. The work entailed in this thesis examines reproductive and detoxification responses in mice. Successful selection in this small animal model might provide an approach to ameliorating fescue toxicosis in cattle.

Reproduction and Enzyme Detoxification Activities in Mouse Lines Selected for Response to Fescue Toxicosis

ABSTRACT: In previous work, one mouse line was selected for resistance to fescue toxicosis (R) and a second line was selected for susceptibility (S) based upon reductions in post-weaning growth rate caused by an endophyte infected diet. The first objective of the current experiment was to determine whether long term reproduction of S mice was more severely depressed than that of R mice by the toxic diet. The second objective was to quantify glutathione-S-epoxytransferase (GST) and uridine diphosphate glucuronosyl-transferase (UDPGT) activities in livers of R and S dams from the experiment and to determine whether reproduction during continuous cohabitation and liver detoxification enzyme activities were correlated within genetic line x diet groups. Twenty-eight mating pairs per line x diet combination, resistant mice fed a non-toxic diet (R-), resistant mice fed a toxic diet (R+), susceptible mice fed a non-toxic diet (S-) and susceptible mice fed a toxic diet (S+), were cohabited continuously for 36 wk. Effects of the toxin-containing diet on the number of offspring born, the number of pups weaned, and litter weights were detectable ($P < .01$) within the first litters produced. Reproductive variables were more seriously influenced by the toxic diet within the S line than the R line when these measures were compared within four equal time phases. The effects of the toxic diet on reproduction were greatest early in the experiment; by the fourth time phase,

differences among line x diet groups in reproduction, with the exception of litter weight, were not significant. Percentage changes in total reproduction of mice fed the toxin-containing diet, for R and S pairs respectively, were -13 and -28 for total number of offspring born, -10 and -25 for total number of offspring weaned, -13 and -14 for total number of litters produced per pair, and -30 and -42 for weight of offspring weaned. Survival percentage of the pups did not differ among groups. Averaged across diets, GST activities were higher in R line mice (P = .05), but differences in UDPGT activities were not significant. Diet affected neither enzyme activity. Glutathione-S-epoxytransferase activity was correlated with the number of offspring born (- .50), the number of litters produced (- .44), and survival percentage (.40) within the R- group. In R+ mice, GST activity remained correlated with survival percentage (.37). Glutathione-S-epoxytransferase activity was not correlated with reproduction in the S groups, and UDPGT activities were not correlated with reproduction in any groups. Selected lines differed in adaptation to the toxic diet as measured by its effect on several components of long term reproduction. In addition, GST activity differed between R and S line mice and was correlated with three reproductive measures.

Introduction

Tall fescue (*Festuca arundinacea* Schreb.) is a well adapted, hardy cool season grass used extensively as a forage in the southeastern United States. Concomitant with its agronomic benefits, fescue infected with the endophytic fungus, *Neotyphodium coenophialum*, poses several health challenges to cattle. These conditions include fat necrosis, fescue foot, and summer syndrome, which collectively result in large economic losses in the production system (Paterson et al., 1995). Mice, as animal models, require little investment per unit and exhibit measurable reproductive responses to endophyte-infected fescue in the diet (Zavos et al., 1987; Zavos et al., 1990; Godfrey et al., 1994).

As described by Hohenboken and Blodgett (1997), ICR mice (Harlan Sprague Dawley Inc., Indianapolis, IN) in our laboratory have undergone seven generations of divergent selection for the impact of a toxin-containing diet on post-weaning gain, resulting in susceptible (S) and resistant (R) lines. Detoxification activities of four liver enzyme families were also examined. Genetic line, diet, and their interaction did not significantly alter cytochromes P450 or b5 enzyme activities. However, R mice showed higher glutathione-S-epoxytransferase (GST) levels than S mice, regardless of diet consumed. These liver enzyme levels were highest for both lines at weaning. In adult male mice fed the toxic diet, uridine diphosphate glucuronosyl-transferase (UDPGT) activity was higher in R mice than S mice. In weanlings, UDPGT activity was higher in R mice

than S mice, increased with age in the R line mice, but decreased with age in the S line.

The current experiment was conducted to determine whether long term reproduction of R mice was less severely depressed by exposure to the toxic diet than reproduction of S contemporaries. Did selection create divergence in adaptability to the toxic diet, as measured by the total reproductive ability of continuously mated pairs? Secondly, are UDPGT and GST detoxification activities correlated with differences in reproductive abilities within genetic line x diet groups?

Materials and Methods

Offspring of generation 12 from both lines were weaned at approximately three weeks of age from dams that had not consumed toxin-containing diets and were randomly assigned four like-sexed mice per cage for a four week growing period. Within each line, half the cages were provided a finely ground and thoroughly mixed toxin-containing diet (+) composed of one half rodent food (Harlan Teklad 7001, Harlan Sprague Dawley, Madison, WI) and one half endophyte-infected KY-31 fescue seed. Remaining cages were provided a ground and mixed non-toxic diet (-) of one half rodent food and one half certified endophyte-free Forager fescue seed. This divided the population into four treatments: resistant mice fed a non-toxic diet (R-), resistant mice fed a toxic diet (R+), susceptible mice fed a non-toxic diet (S-), and susceptible mice fed a toxic diet (S+). At

approximately eight weeks of age, 28 males and 28 females from each genetic line x diet combination were randomly sampled and mated (except that brother/sister matings were prohibited), for a total of 112 pairs.

One mated pair from each treatment combination was then assigned at random to one of four adjacent cages in each of 32 blocks in two standard rodent cage racks. Cages were 15-cm x 21-cm x 29-cm transparent plastic with nipple-flow waterers and paper fiber bedding. The room temperature averaged 24°C and fluorescent lighting was provided from 0700 to 1900 daily.

Determination of reproductive abilities

Pairs were maintained in continuous cohabitation for 36 wk. Mice were checked daily for new litters and the number of pups born, including dead pups, was recorded. Litters were weaned and pups individually weighed and killed at an intended 18 d post-partum (range 17 to 21 d). Pairs that failed to produce pups within 56 d of their initial paring or of their previous parturition were eliminated from the experiment.

The total numbers of pups born (tborn), of pups weaned (twean), and of litters produced (tnum) by each pair were recorded. In addition, weaning weights of each litter produced per pair were totaled (twght) after adjustment to a standard weaning age of 18 d post-partum. The adjustment was accomplished by dividing the litter weight by weaning

age, multiplying that value by the deviation of weaning age from 18, and adding the product to the actual litter weaning weight. A fifth variable, survival percentage, was computed by dividing twean by tborn.

Reproductive variables were also examined by dividing the study into four 57 or 58 d phases, beginning from the earliest day of birth; and the number of pups born, number of pups weaned, umber of litters produced per pair, and adjusted litter weights were summed within each time period. This categorization allowed examination of line, diet and interaction effects over time; changes in reproductive measures between time periods may or may not have been equal among line x diet groups.

The first reproductive interval (int1) was calculated by computing the time interval from each pair's date of initial cohabitation to the date of first parturition. All other intervals (int2 – int8) were calculated by computing the days between subsequent parturition dates.

Determination of liver enzyme activities

After 36 weeks cohabitation, sires and dams were killed by carbon dioxide asphyxiation and livers were harvested, individually bagged and identified, and stored on ice for a short time (10 to 90 min). The livers were then stored at -70°C for approximately 18 mo. Ninety-one livers from female mice were divided into seven groups

containing nearly equal numbers of livers from R+, R-, S+, and S- mice, and each of the seven groups was processed on a different day.

Glutathione-S-epoxytransferase activity was measured based on methodology provided by Kaplowitz et al. (1975) and described in Hohenboken and Blodgett (1997). The procedure was modified in this experiment to include the use of a microplate reader (Spectramax 250, Molecular Devices Corporation, Sunnyvale, CA). Using .05 m*M* 1,2-epoxy-(p-nitrophenoxy) propane as a substrate, a 300 mL solution containing 10 m*M* GST, 25 mL liver cytosol, and 175 mL sodium phosphate buffer (pH 6.5) was incubated at 37°C for 11 minutes in a microplate reader. Enzyme activity was determined as the change in absorbance at 360 nm over the 11 min incubation (ε_{360} = .51 m•M^{-1}•cm^{-1}).

Uridine diphosphate glucuronosyl-transferase activity was determined based upon procedures described by Dutton et al. (1981) and modified by Hohenboken and Blodgett (1997). No further modifications were made in this project. Activity of UDPGT was determined as the change in absorbance at 550 nm (ε_{550} = 3.9 x 10^4•M^{-1}•cm^{-1}).

Statistical analysis

Data for reproductive variables and liver enzyme activities, and correlations between the two were analyzed using the GLM procedure in SAS (1985). Reproductive variables were analyzed with a model including sources of variation for genetic line, diet,

line x diet, block, and residual. Data for GST and UDPGT were analyzed with a model including sources of variation for genetic line, diet, line x diet, day or block, and residual. Block (four adjacent cages in a standard laboratory rodent cage rack) was dropped from both final models because its influence on reproductive traits and liver enzyme activities was not significant. Correlations between reproductive measures and GST and UDPGT activities were computed within each line x diet treatment group.

Results and Discussion

Early Reproduction

As indicated by Table 1, reproductive measures either differed significantly between lines during the first parity (int1, litwt1) or became significantly different by the second (nborn2, nwean2). The toxic diet had a detrimental impacted on all measured reproductive traits in both the first and the second parity. As shown in Table 2, genetic line x diet effects were significant for most traits during the first parturition; nborn1, nwean1, and litwt1 were severely decreased in S+ mice compared to S- pairs but not in R+ compared to R- pairs. However, by the second parity, nwean2 and litwt2 were not significantly influenced by the line x diet interaction.

The toxic diet influenced reproduction early in the experiment. However, nwean

and litwt may be more resilient aspects of reproduction than nborn because they began to recover by the second littering. This may be explainable by dividing these reproductive measures into pre-partum and post-partum traits. The number of pups born in a litter, a pre-partum trait, is influenced by male and female fertility, conception, and embryonic survival. Zavos et al. (1990) found that endophyte-infected diets affected male fertility. In addition, toxic diets reduced fertilization and embryonic viability in female mice (Zavos et al., 1988b). Post-partum traits, nwean and litwt, are influenced by maternal factors such as lactation, which is detrimentally impacted by toxin containing diets (Zavos et al., 1988c). However, the progeny may have more influence over their own survival than they would have had pre-partum. Zavos et al. (1987) observed significant differences between mice fed toxic and non-toxic diets in the number of pups produced per litter but no difference in the total weight of pups weaned per litter.

The difference between the R and S lines in int1 and int2 (Table 1) may be related to the difference in mature size of mice between lines. In previous work, T. Howell and W. Hohenboken (unpublished data) found that susceptible mice were larger than the resistant line mice at maturity, possibly as an inadvertent result of our selection criterion. Gestation within larger-framed breeds of cattle tends to be longer than the gestation within smaller-framed breeds (USMARC, 1990) and perhaps a similar event is occurring here. In addition, although S mice delivered significantly fewer pups than R mice during the first two parturitions (Table 1), the two lines lost proportionally the same number of

pups between birth and weaning and the average individual pup weight was approximately equal.

Reproduction divided into four time phases

Mice were continuously cohabitated in order to determine if genetic line x diet interaction effects would become more dramatically apparent under longer-duration dietary and biological stress. The least squares mean of number of pups born per mouse pair of each line x diet group within each time phase is illustrated in Figure 1. Resistant mice fed the non-toxic diet showed a consistent linear decrease in pup production over time. All other line x diet combinations showed similar, though less consistent, decreases. Dividing the data into four time phases shows that the impact of the toxic diet on S mice was greatest during the earliest few weeks of the experiment. During phase IV the number born within each line x diet group did not differ significantly. The number of pups weaned, given in Figure 2, and the number of litters produced, illustrated in Figure 3, exhibited expectedly similar results. Adjusted litter weights, in Figure 4, followed a similar pattern, but R- mice consistently differed from all other line x diet combinations.

The effect of time on reproduction

Advancing age, a biological stressor, could also influence reproduction. However, though reproductive measures changed over time, the patterns of change were similar among genetic line x diet groups; there was no genetic line x diet x time interaction. As an example, Figure 5 shows the length of intervals between adjacent parities. Parturition intervals remained relatively constant between parities two and six and they did so similarly for R-, R+, S-, and S+ groups. Fewer mice are left within each line x diet group as the experiment progressed and the pooled standard error ranged from 1.2 d in the first interval to 1.8 d in the seventh. Conversely, Zavos et al. (1987) showed an increase in parturition intervals when pairs were fed a toxic diet during continuous breeding.

Measures of total reproduction

As shown in Table 3, averaged across diets, the R line was significantly superior to the S line for all reproductive traits. Also, averaged across lines, the + diet caused significant and substantial reductions in all traits except pup survival percentage. Diets containing endophyte-infected fescue are known to influence both male and female reproduction (Zavos et al. 1987; Zavos et al. 1990; Godfrey et al. 1994), though it was not possible to tell in this study the extent to which males versus females were affected.

As shown in Table 4, line x diet interactions did not achieve statistical significance at $P<0.05$ for any of the cumulative reproductive traits, but this may be misleading. As shown in Figures 6-9, the percentages of reduction in a trait caused by the + diet, for R and S mice, respectively, were 13 and 28 for total number of pups born, 10 and 25 for total number of pups weaned, 13 and 14 for total numbers of litters produced, and 30 and 42 for total weight of pups weaned. This suggested better adaptability of R than S mice to the + diet. For pup survival percentage, however, there was no evidence of line x diet interaction.

Liver detoxification enzyme activities

Least square means for the main effects of line and diet on liver enzyme activities and their significance levels are given in Table 5. Table 6 shows genetic line x diet effects on liver activities, as well as pooled standard errors and significance levels. The effects of diet and the genetic line x diet interaction were not significant for GST activity. However, averaged across diets, R mice had higher GST activity than S mice. These results are in concurrence with Hohenboken and Blodgett (1997), who showed that GST levels in R line mice were higher than those in the S line in weeks 1-5 post weaning. Uridine diphosphate glucuronosyl-transferase activity did not significantly differ between lines or diets, or within line x diet groups. Hohenboken and Blodgett (1997) found that R+ mice

had higher UDPGT activity than S+ mice during one experiment, but the results were reversed in a second experiment. In a third experiment, Hohenboken and Blodgett observed that R mice had higher UDPGT levels than S mice and that both groups had increased activity between 1 and 3 wk post-weaning. However, UDPGT activity increased in R mice and decreased in S mice after that time.

Glutathione-S-epoxytransferase and UDPGT activities were expected to be higher in R than S mice based upon previous studies of this mouse population. This study showed that GST activity did differ between genetic lines, but activities of UDPGT showed no differences. Hohenboken and Blodgett (1997) compared mice during a 1-5 wk post-weaning period. It is possible that GST, and not UDPGT, is the major enzyme system that detoxifies components in toxic fescue seed in adult mice. It is more likely that harvesting livers and measuring enzyme activities from mice at younger ages may have provided significant correlations, however. Zhu et al. (1995) showed the extent of UDP-glucuronyltransferase induction with sodium phenobarbital differed with age in female rats. Lundqvist and Morgenstern (1995) found that the ability of *N*-ethylmalemide to induce glutathione transferase activity in female rats was significantly lower early (35-100 d) and late (300-550 d) in life. Ideally, the mouse livers analyzed in this study should have been collected before the mice reached 300 days of age, but this could not be accomplished in the study of genetic line and dietary effects and interactions on long term reproduction.

The day of liver enzyme extraction and activity measurement was significant (P = .02) for both GST and UDPGT enzymes. Glutathione-S-epoxytransferase activities measured on days six and seven differed from assays performed on days one through four. Day one UDPGT activities differed from days two through seven and days three and seven differed from day five.

Correlations between liver enzyme activity and reproductive measures

Correlations between reproductive traits and liver enzyme activities within R+, R-, S+ and S- groups are given in Table 7. With few exceptions, liver enzyme activities were not correlated with measures of reproduction. In R- mice, GST was moderately negatively correlated with tborn and tnum. Within the R line, regardless of diet, GST was moderately correlated with survival. Correlations between GST and UDPGT activities within the R-, R+, S- and S+ groups were .21 (P = .30), .16 (P = .49), .20 (P = .41), and .29 (P = .19), respectively.

Conclusions

Resistant mice were better able than their S contemporaries to cope with the challenge of consuming a toxic diet during continuous cohabitation. Within the first two

parturitions, R+ mice were able to produce and wean more offspring than S+ mice. Consequently, litter weights for R+ mice were also greater than for S+ mice. Impacts of the toxic diet were greater on S than R mice, and these effects were most apparent within the early part of the experiment. Though R mice tended to show greater resistance to the toxic diet than S mice, as measured by total reproduction, the differences between lines occurred mostly at the beginning of the experiment. Selection created divergence in adaptability to the toxic diet.

Glutathione-S-epoxytransferase activity was higher in R than S mice, regardless of the diet fed. However, UDPGT activity was similar among line, diet, and line x diet treatment groups. Low correlations between reproductive traits and liver enzyme activities suggested that other physiological mechanisms may be important in determining resistance or susceptibility to toxic challenge.

Implications

Endophyte-infected fescue seed in the diet impaired reproductive performance in mice. However, mice selected for resistance to the condition were better able to tolerate toxic challenge. Selection in beef cattle for resistance to fescue toxicosis might ameliorate economic and biological hardships imposed by the disease. Previous work suggested that GST and UDPGT activities enable R line mice to better cope with the impact on growth

caused by toxic fescue seed diets. Elevated GST activity remained detectable in adult female mice of the R line, but within line x diet groups did not seem to provide a reproductive advantage to these dams when they were challenged with a toxic diet. Additional studies are needed to find other physiological coping mechanisms to mitigate the effect of fescue toxicosis.