PENTOBARBITAL SLEEP TIME IN MOUSE LINES SELECTED FOR RESISTANCE AND SUSCEPTIBILITY TO FESCUE TOXICOSIS

by

Kimberly Ann Arthur

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.A. Emmerson **D.M. Moore**

January, 2002
Blacksburg, VA

Key Words: ***Festuca arundinacea,*** **Mice, Enzyme Activity, Induction, Anesthesia**

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Committee Chairman: William D. Hohenboken
Department of Animal and Poultry Sciences
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(ABSTRACT)

In previous work with mouse lines selected for resistance (R) and susceptibility (S) to fescue toxicosis, R mice had higher activities of Phase II liver enzymes glutathione S-transferase and uridine diphosphate glucuronosyl-transferase than S mice. Objectives of this study were: 1. to determine whether selection for toxicosis response had also caused divergence between lines in hepatic Phase I enzyme activity (as assessed by sleep time following sodium pentobarbital anesthesia), 2. to determine whether sleep time differences between lines were modulated by fescue toxins or enzyme inducers in the diet, and 3. to determine whether sleep time differences among individual mice were correlated with the impact of a toxin-containing diet on their post-weaning growth.

In experiment 1, five dietary treatments were assigned to 24 male mice in each line: rodent food control, E+ (50% endophyte-infected fescue seed, 50% control), E+P (E+ with 1000 ppm phenobarbital), E- (50% endophyte-free fescue seed, 50% control), and E-P (E- with 1000 ppm phenobarbital). After four weeks on these diets, mice were challenged with a sleep time test. All mice were then switched to a pelleted rodent food diet. Each mouse then received a second sleep time test, a random 1/4 of the population after one, two, three, and four weeks on the standard diet. Results demonstrated that, regardless of dietary treatment, R mice had a shorter sleep time than S mice, suggesting higher activity of liver Phase I microsomal enzymes. Mice that were fed phenobarbital had significantly shorter sleep time than those whose diets did not include this microsomal enzyme inducer. Time interval between the first and second sleep time did not significantly impact the second sleep time, confirming line differences in the absence of toxins and inducers and with advancing age.

In experiment 2, male and female R and S mice were fed an E- diet for 2 weeks, then an E+ diet for 2 weeks, followed by a pelleted rodent food diet for 2 weeks. Mice were then administered a sleep time test. Their growth rate response to fescue toxicosis was quantified as the proportional reduction in gain during two weeks on the E+ diet, compared to gain on E- during the previous two weeks. Sleep time was significantly influenced by line but not by sex or the line x sex interaction. As in Experiment 1, S mice slept longer than their R counterparts. The residual correlation between reduction in gain associated with the E+ diet and sleep time was only 0.04. Thus, under these experimental conditions an individual animal's Phase 1 enzyme activity did not predict how severely its growth rate would be depressed by a toxin-containing diet.

Based upon these and previous studies, divergent selection for toxicosis response in mice was successful partially by causing divergence between lines both in Phase I and Phase II liver detoxification enzyme activities. If a heritable, practical, and economical criterion could be identified to quantify such differences in livestock species, then selection for toxicosis resistance might contribute to the solution of this important problem for American agriculture.

Dedication

This thesis is dedicated to my family:
My parents, Joyce and Warren Arthur
My brothers, Kevin and Douglas Arthur
My grandmother, Viola Dales
And my best friend, John Ziercher
for their constant love and continued support in my endeavors.

Acknowledgements

I am grateful to many people that have lent their assistance and support throughout this study:

Dr. W. D. Hohenboken - for teaching me the difference between science and good science, and for his patience throughout my program and preparation of this manuscript. I like to think that I was lucky to have had the opportunity to work with such a scientist before his retirement and not that I was the impetus for it.

Larry Kuehn – for "reminding" me how much I enjoy statistics and for his outstanding technical assistance.

Drs. D.A. Emmerson and D. M. Moore - for their advice, support, and willingness to serve on my graduate committee.

Dr. P.B. Siegel – for sharing a pitcher now and then, and demonstrating his exemplary weaving skills…an admirable blanket of accomplishments including well-balanced patterns of family, friends, life, and science.

And especially my family:
My parents – for providing me with love, words of wisdom, and a foundation of strong values upon which I could build my education.

My brothers – for believing in me, keeping me grounded, and being my friends.

My grandmother – for our Sunday afternoon chats and her moral support, prayers, and never-wavering confidence in me.

John Ziercher –for his encouragement, love, and tireless support; for helping every time in every way; and for instilling the importance of a good (great) work ethic. I can finally get rid of all those papers!

Table of Contents

Page

Abstract……..…….....ii
Dedication…….…….…….iv
Acknowledgements…….…….v
Table of Contents……....vi
List of Tables…….vii
List of Figures……...viii
Introduction……..1
Literature Review…….…3
Tall Fescue and its Effects on Livestock…….3
Alkaloids……..…4
Biotransformation…….…....8
Enzyme Induction……..12
Barbiturates……13
Genetic Variation in Toxicosis Resistance……14
Materials and Methods…….…..19
Experiment 1…….…….19
Experiment 2…….…….22
Results…….…...24
Experiment 1…….…….24
Experiment 2…….…..…30
Discussion…….… 34
Conclusions…….……...37
Implications…….……...38
Literature Cited…….….39
Vita…….…45

List of Tables

Table 1. Experiment 1: Numbers of mice not completely anesthetized per subclass of 24 individuals……..…24

Table 2. Experiment 1: Mean squares and P-values from analysis of variance of sleep latency and sleep time 1…….24

Table 3. Experiment 1: Least squares means (± standard error) for line and dietary treatment effects on sleep time following sodium pentabarbitol anesthesia……..25

Table 4. Experiment 1: Mean squares and P-values from analysis of variance of cumulative weight gains while on the experimental diets…….……27

Table 5. Experiment 1: Mean squares and P-values from analysis of variance of sleep time 2……..…28

Table 6. Experiment 1: Least squares means (± standard error) for line, dietary treatment, and time interval effects on the second sleep time measurements on the mice…….29

Table 7. Experiment 1: Residual correlations between sleep time 1 and sleep time 2 for mice whose second sleep time followed their first by varying intervals of time……...30

Table 8. Experiment 2: Mean squares and P-values from analysis of variance of sleep latency and sleep time……30

Table 9. Experiment 2: LS means and standard errors for main effects on sleep time....31

Table 10. Experiment 2: Mean squares and P-values from analysis of R_M, E-GN, E+GN, and SGN……..…31

Table 11. Experiment 2: LS means and standard errors for R_M……...32

List of Figures

Figure 1. Metabolism of tall fescue……..11

Figure 2. Experiment 1: Least squares mean sleep time for line by dietary treatment groups…….……26

Figure 3. Experiment 1: Cumulative weight gain during 4 weeks on experimental diets. Week 0 is weaning. Standard errors of diet subclass means were 0.17, 0.23, 0.36, and 0.38 for wk 1-4 respectively……..27

Figure 4. Experiment 2: Mouse growth. Wk 0 is weaning. Wk 0-2=E-, Wk 2-4=E+. Wk4-6=Standard laboratory rodent food. Standard errors were 0.23, 0.15, and 0.20 for wk 2, 4, and 6 respectively…….33

Introduction

Tall fescue (*Festuca arundinacea*) is a hardy, cool season, perennial bunch grass. It covers over 15 million hectares in the United States, providing pasture for an estimated 8.5 million beef cattle (Ball et al., 1993) and 688,000 horses (Ball et al., 1993; Hoveland, 1993). Tall fescue's popularity is based on its ease of establishment, wide geographical adaptation, long grazing season, and tolerance to abuse. More than 90% of fescue stands are infected (Siegel et al., 1984 a, b) with a mutualistic endophytic fungus presently identified as *Neotyphodium coenophialum*. Within these stands, over 60% of individual plants typically contain the fungus (Siegel et al., 1984 a, b). The fungus lives between the plant cell walls and produces a number of alkaloids and other toxins thought to cause poor performance in livestock. Fescue toxicosis, the most common manifestation of ingestion of endophyte-infected (E+) fescue, causes annual economic losses in livestock of at least $1.1 billion in this country alone (Hoveland, 1993; Cross, 1997).

Many attempts have been made to alleviate the impact of fescue toxicosis on livestock production, none of them universally effective. Selection of cattle (Lipsey et al., 1992) for the ability to tolerate toxins from E+ fescue is a potential genetic solution. However, before effective selection and mating systems can be designed, it is necessary to study the biology of host resistance to the toxins.

Mice have been used as animal models for studying fescue toxicosis, because they require little investment per unit, and they exhibit measurable responses to E+ fescue (Zavos et al., 1990). In mice divergently selected for the impact of an E+ containing diet on post-weaning gain, Wagner et al. (2000) reported that reproduction in the susceptible line was more severely depressed by the toxin-containing diet than was reproduction in the resistant line. Those same lines were used by Hohenboken and Blodgett (1997) when they found that mice divergently selected for eight generations for resistance (R) or susceptibility (S) to postweaning growth depression from E+ fescue seed in the diet exhibited differences in Phase II liver enzyme activities.

The objective of this study was to determine whether past selection for response to fescue toxicosis in these mouse lines had caused divergence between lines in hepatic Phase I detoxification function as assessed by sleep time following sodium pentobarbital anesthesia. Experiment 1 sought to answer the following questions:

- When fed a diet containing E+ fescue seed, E- (non-endophyte infected) fescue seed, or neither, do resistant and susceptible line mice differ in liver function as assessed by sleep time following sodium pentobarbital anesthesia?
- Does prior induction of hepatic Phase I detoxification enzyme systems by ingestion of phenobarbital modulate line differences in sleep time in response to subsequent sodium pentobarbital anesthesia?

Experiment 2 sought to answer the questions:

- Were line differences in sleep time following sodium pentobarbital anesthesia found in Experiment 1 expressed under a different dietary regimen?
- Was sleep time following sodium pentobarbital anesthesia of individual animals correlated with the impact of E+ fescue in the diet on their post-weaning growth (the trait originally used as the criterion for selection of toxicosis response)?

Literature Review

Tall fescue and its endophyte, *Neotyphodium coenophialum* (formerly known as *Acremonium coenophialum*) are mutually symbiotic. From the grass, the fungus derives a medium in which to grow, all of its nourishment and, through infection of fescue seed, a means to disperse. Plants infected with endophyte are more drought and parasite tolerant than non-infected plants (Joost, 1995). Unfortunately the toxins produced by tall fescue in association with *Neotyphodium coenophialum* create pathological conditions in grazing animals (Thompson and Stuedemann, 1993).

This literature review covers effects on livestock, identification of alkaloids, biotransformation, enzyme induction, barbiturates, and genetic selection as they pertain to tall fescue toxicosis in livestock.

Tall Fescue and its Effects on Livestock

Fescue toxicosis causes functional changes in alpha-2 adrenergic receptors in blood vessels (Oliver et al., 1998), thermoregulatory disturbance, altered lipid metabolism, and impaired reproduction in grazing animals. Common manifestations are increased body temperature, reduced body weight gain, reduced serum cholesterol and prolactin concentrations, and lowered alkaline phosphatase activity. Decreases in serum cholesterol concentrations and alkaline phosphatase activity develop only after animals have ingested endophyte-infected (E+) fescue for prolonged periods (Lipham et al., 1989).

Cattle commonly suffer from one of three forms of the disease: summer slump, fescue foot, or fat necrosis. Summer slump is a form of hyperthermia in which cattle suffer from an elevated body temperature manifested as reduced feed intake, poor growth, weight loss, elevated respiration, and increased susceptibility to heat stress. Fescue foot, a form of hypothermia, is a gangrene of the extremities. It is caused by

peripheral vasoconstriction due to the effects of ergot alkaloids on smooth muscle contraction, leading to reduced blood flow and anoxia. Finally the tissue is destroyed and sloughs off. Fat necrosis is generally seen as nodules in the abdominal cavity that vary in size, shape, and consistency. Fat necrosis may be related to the vasoconstriction observed in fescue foot where the vasoconstriction leads to febrile conditions (as seen in summer slump) that initiate lipolysis (Rumsey et al., 1979). Fat necrosis is generally seen in cattle that have grazed on E+ tall fescue for several seasons or on pastures that have been heavily fertilized with nitrogen or poultry litter (Rumsey et al., 1979; Stuedemann et al., 1985).

Alkaloids

Several alkaloids have been isolated from E+ tall fescue. Alkaloids contain nitrogen and a heterocyclic ring. They are usually bitter and most are toxic. They are synthesized from amino acids. There are two major sources of alkaloids, those produced by fungi that infect the seed heads of grasses and those that are produced by endophytic-fungi. The first type are the diazaphenanthrene alkaloids, including perloline and perlodine. Although most toxicological problems associated with tall fescue are thought to be caused by endophyte-produced alkaloids, other alkaloids also appear to contribute to the ADR "ain't doing right" response seen in fescue toxicosis. Perloline has physiological effects on animals (Boling et al., 1975). After parenteral administration of this alkaloid, convulsions, muscle incoordination, increased pulse and respiration rate, mild photosensitization, and coma occur in sheep. Boling et al. (1975) demonstrated that administration of 0.5% perloline to lambs reduced the digestibility of protein and cellulose and reduced nitrogen retention. Production of volatile fatty acids in the rumen also was reduced, and body temperatures of the perloline-fed lambs tended to be higher. Inhibition of rumen cellulolytic organisms by perloline was also shown.

The endophyte, *Neotyphodium coenophialum,* upon infection of grass, produces a wide variety of toxins including ergopeptine and loline alkaloids which are thought to be

responsible for causing fescue toxicosis. Both are found only in E+ fescue, suggesting that they are of endophytic origin. The roles of these alkaloids are not definitively known, but individually or in combination they may contribute to fescue toxicosis. The endophyte can synthesize peptide ergot alkaloids (mainly ergovaline), lysergic acid amide, clavine ergot alkaloids, pyrrolizidine alkaloids, and pyrrolpyrazine alkaloids such as peramine (Porter, 1995).

Ergopeptine Alkaloids. Ergopeptines have been the most studied ergot alkaloids associated with fescue toxicosis (Strickland et al., 1993). These alkaloids induce behavioral, gangrenous, reproductive, and weight gain problems in livestock. Peptide alkaloids are physiologically active. Although they disappear rapidly from blood and tissue with a high first pass clearance through the liver (Moubarak et al., 1996), their physiological effects persist for long periods of time. The mode of action of ergopeptine alkaloids is through an inhibitory effect on prolactin secretion by the pituitary, by activating D_2 dopamine receptors in pituitary lactotrophs. The ergoline found in ergot peptide alkaloids is structurally similar to norepinephrine, dopamine, and seratonin (Berde, 1980: Strickland et al., 1993), which may account for its ability to alter vasoregulatory mechanisms and prolactin levels via dopamine D_2 receptors. Ergovaline, the principal and most abundant of the ergopeptines present (Lyons et al., 1986) in E+ tall fescue (up to 90%), may also be the most toxic. Ergovaline content of E+ fescue increases with nitrogen fertilization and plant maturity.

Unfortunately ergovaline is not commercially available and has not been isolated in sufficient quantities for use in animal studies. Larson et al. (1999) found that synthesized ergot alkaloids are acceptable alternatives in the D_2 dopamine receptor system to stimulate receptor binding responses and cyclic AMP inhibition that occurs after administration of naturally occurring ergot alkaloids extracted from tall fescue. In addition to interacting with dopamine receptors, several ergot alkloids may produce dopaminergic effects by increasing the release of dopamine from central nerve cell endings (Rowell and Larson, 1999). Administration of bromocriptine, a synthetic ergot

alkaloid, to pregnant mares produces similar adverse reproductive effects as does ingestion of E+ tall fescue (Ireland et al., 1991).

Ergot alkaloids have a direct stimulatory effect on smooth muscle, causing vasoconstriction and elevated blood pressure. However these alkaloids do not appear to affect intestinal motility (Rotter and Phillips, 1991). Bovine dorsal pedal veins contracted when exposed *in vitro* to various ergot alkaloids but not when exposed to pyrrolizidine alkaloids (PA) (Solomons et al., 1989).

Loline Alkaloids. The loline alkaloids are saturated PA with the major ones in E+ fescue being N-acetyl and N-formyl loline. These alkaloids lack the 1,2 double bond that is characteristic of hepatotoxic pyrroloizidine alkaloids. The role of loline alkaloids in fescue toxicosis has been controversial, as the major causative agent seems to be the ergopeptine alkaloids. Their activity as D_2 dopamine receptor agonists is minor compared to that of ergovaline (Strickland et al., 1994; Larson et al., 1999). However, loline alkaloids may have mild vasoconstrictive properties (Strickland et al., 1993) which may contribute to fescue foot and hyperthermia syndromes. Studies with loline alkaloids alone indicate that they do suppress *in vitro* prolactin secretion by rat pituitary cells (Strickland et al., 1993) and may have a slight inhibitory effect on prolactin release from the pituitary (Strickland et al., 1994; Larson et al., 1999). While Jackson et al. (1996) found that loline alkaloids may have depressed feed intake, those alkaloids did not reduce serum prolactin concentration or otherwise impact performance in rats.

The major loline alkaloid (70-80% of total) in E+ tall fescue is N-formyl loline, with 15-20% N-acetyl loline and minor quantities of other lolines (Yates et al., 1990) making up the remainder of the loline content. N-acetyl and N-formyl loline are converted to loline in the rumen by microbial action (Westendorf et al., 1993). Loline is then absorbed from the rumen in a process call biotransformation.

Pyrrolizidine Alkaloids. Although PA are present in tall fescue at lower concentrations than the previous alkaloids, their presence may still be a contributing factor to the pathological conditions associated with fescue toxicosis. Long-term ingestion of PA causes chronic disease in animals. Signs and symptoms include poor growth, cumulative liver degeneration, reproductive failure, and a general slow wasting away (Bull et al., 1968). There is conjecture that PA may be partially responsible for some contractile activity of vasculature experienced in fescue toxicosis.

Pyrrolizidine alkaloids are hepatotoxins (Mattocks and Jukes, 1986), impairing liver function by reducing the liver's ability to deaminate amino acids and synthesize urea from ammonia. The liver storage of copper is increased and zinc stores are decreased in the presence of pyrrolizidine alkaloids, thus affecting metabolism of proteins, vitamins, and minerals. Vitamin A levels are also affected, leading to a reduction in retinol binding protein. Protein synthesis is inhibited because PA become cross-linked with DNA strands, interfering with DNA replication and RNA synthesis. Pyrrolizidine alkaloids inhibit the prophase stage of mitosis. Hayek et al. (1991) reported that PA inhibited *in vitro* mitogen-stimulated proliferation of bovine and murine lymphocytes. Pyrrolizidine alkaloids used in combination had no effect on *in vitro* rat pituitary prolactin secretion (Strickland et al., 1992).

Pyrrolizidine alkaloids are a product of secondary metabolism in animals. Hepatic oxidation of these PA compounds results in metabolites that are more toxic than the parent compound. In the case of PA, metabolites become chemically reactive pyrrole derivatives (pyrrolic dehydro-alkaloids) in a process called biotransformation. Animals that are resistant to the toxic effects of PA, sheep, guinea pigs, and Japanese quail for example, have a much lower rate of pyrrole production than susceptible species such as cattle, horses, and rats (Cheeke, 1994).

Biotransformation

The intensity and duration of effect that foreign compounds (xenobiotics) have on animals is determined in large part by the animal's rate of metabolism. Although drugs and other foreign compounds are metabolized in tissues throughout the body, the liver is the most active site (Swick, 1984). Liver tissue has high levels of xenobiotic metabolizing enzymes. Absorbed substances are taken to the liver by the portal circulation before entering the general circulatory system of the body. Drug metabolizing enzymes are located primarily in the endoplasmic reticulum of cells and are classified as microsomal enzymes. The hepatic microsomal drug metabolizing system is important in determining the resistance of animals to drugs and toxins (Smith et al., 1980).

Most toxicants are absorbed in a lipid soluble form, enzymatically metabolized (biotransformed) in the tissue, and excreted as water-soluble metabolites in the urine. The biochemical end result of this process is to change the polarity of the molecule (toxicant) and allow its translocation within the body. This translocation includes passage through membranes, which occurs when polarity is decreased, and increased aqueous solubility and urinary excretion when polarity is increased. The rate of absorption is determined largely by lipid solubility. Non-ionic compounds are more readily absorbed than ionized substances because they are more lipophilic. Most toxicants are absorbed by simple diffusion. The most toxic alkaloids have low water solubility and a low degree of ionization, whereas less toxic alkaloids have high water solubility and a high degree of ionization (Smith and Reynard, 1992).

Biotransformation may affect binding and storage of drug molecules within the body and may either increase or decrease the toxicity of the absorbed toxin. This metabolic process is largely carried out by nonspecific microsomal liver enzymes known as mixed function oxidases (MFO). These enzymes are capable of metabolizing many diverse compounds and respond quickly to the presence of dietary toxins with an increased level of activity. This process, whereby the presence of a toxicant induces

increased activity of the enzymes that detoxify it, is known as enzyme induction (Smith and Reynard, 1992).

The MFO system has several components: cytochromes P450, NADPH, NADPH cytochrome P450 reductase, and phosphatidylcholine. Cytochromes P450 are terminal oxidases of the MFO system. They are bound to hemoprotein-containing membranes. The quantity and efficacy of these membrane bound enzymes varies with species, age, nutritional status, health status, and environment. The MFO system accelerates a reaction that results in more polar, water-soluble metabolites (Parke et al., 1991). There are many forms (isozymes) of cytochrome P450. Some animal species differences in susceptibility to toxins may be due to differences in cytochrome P450 isozyme activities (Ioannides and Parke, 1990; Smith 1991).

The reactions catalyzed by xenobiotic-biotransforming enzymes are divided into two types, Phase I and Phase II. In Phase I, reactive functional groups are added or exposed. Phase I reactions occur in microsomes, mitochondria, and the soluble fractions of tissue homogenates. The MFO system is the primary mediator of Phase I reactions. Phase I reactions include oxidation, reduction, and hydrolysis. Most of the enzymes catalyzing these reactions are present in the smooth endoplasmic reticulum. Phase II reactions may involve conjugation of the metabolites produced in the MFO-mediated Phase I reactions and include glucuronidation, sulfation, methylation, acetylation, and mercaptic acid formation (Smith and Reynard, 1992).

There are some important differences between Phase I and Phase II reactions. Phase I reactions generally produce only a small increase in membrane hydrophilicity whereas Phase II reactions result in a large increase in membrane hydrophilicity. This becomes important during excretion. The lipophilicity that enables many xenobiotics to be absorbed through the skin, lungs, or GI tract later becomes an obstacle to elimination, because lipophilic compounds can be reabsorbed. Consequently, the elimination of xenobiotics often depends on biotransformation, i.e., conversion to a more water-soluble

chemical. Without biotransformation, lipophilic xenobiotics would be excreted from the body very slowly and eventually could overwhelm and kill the organism. Biotransformation can be altered by factors including changes in diet, hormones, drugs and pesticides, age, and species of animal (Swick, 1984).

Most Phase I metabolism is catalyzed by the cytochrome P450 enzymes. Recombinant DNA technology has provided the identification of over 70 distinct cytochrome P450 genes in various species (Sipes and Gandolfi, 1991). Various cytochrome P450 forms in the liver are regulated by pituitary and gonadal hormones (Waxman and Azaroff, 1992) as well as by drugs and other foreign compounds. These enzymes normally biotransform lipophilic drugs, detoxifying the original compounds and making them readily excretable. However, the PA are oxidized into more reactive metabolites (intermediates) that are capable of irreversible covalent binding to nucleic acids or functional proteins within cells (Swick, 1984). An outline of this process is shown in Figure 1.

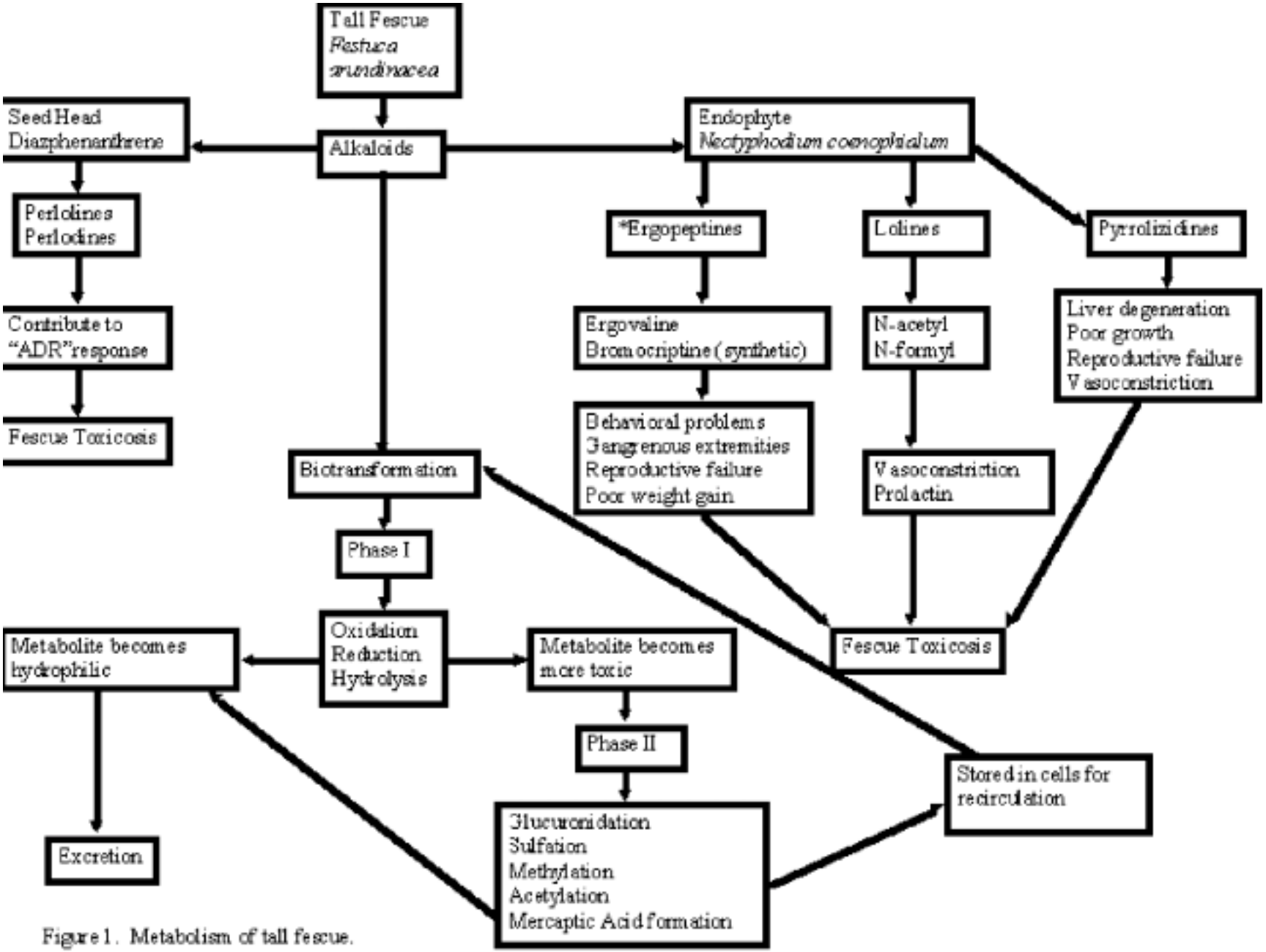

Figure 1. Metabolism of tall fescue.

The most important enzymes in Phase II reactions are glucuronyl transferase, glutathione S-transferase (GST), and sulfotransferase (Sipes and Gandolfi, 1991). Their synthesis can be induced by ingestion of various anti-oxidants (Buetler et al., 1995). Glucuronyl transferase is present in the nucleus and in membranes of the endoplasmic reticulum. Glutathione S-transferase is present both in the cytosol and nucleus of the cell. Sulfotransferase is present as soluble enzyme in the cytosol. Glucuronidation is a major pathway in xenobiotic transformation in all mammalian species with the exception of felids. Conjugation activity in organelles can facilitate the rapid detoxification of activated xenobiotics produced by the cytochrome P450 systems also present there.

Enzyme Induction

Induction of MFO activity requires an increase in concentration of cytochrome P450 and an increase in protein synthesis. These increases can affect xenobiotic biotransformation. Induction takes time and is a reversible process subject to individual variations. This system affects a multitude of chemical reactions. Several inducing agents that modify response to toxicants and drugs are of practical interest to livestock producers. The MFO inducers include barbiturates and steroid hormones. Induction leads to an increased rate of biotransformation and results in decreased drug concentrations, which usually translates to decreased pharmacologic effects of the drugs in animals.

Enzyme induction may or may not be associated with an increase in liver weight and changes in the smooth membrane of the endoplasmic reticulum. Enzyme induction increases RNA synthesis and is prevented by inhibitors of RNA synthesis. Enzyme inducers frequently act to displace drugs from plasma binding sites (Smith and Reynard, 1992). This phenomenon of enzyme induction may be used in the treatment of disease. It is presently being used in humans for the treatment of Cushing's Syndrome and neonatal hyperbilirubinemia. Enzyme induction may be responsible for the development of tolerance to such drugs as barbiturates because these drugs stimulate their own metabolism**.**

Barbiturates

Phenobarbital is a barbiturate occasionally used as an oral sedative agent. Phenobarbital becomes widely distributed throughout the body. However due to it low lipid solubility, it does not distribute as rapidly as most other barbiturates into the central nervous system. It is metabolized in liver tissue (Conney, 1967). Phenobarbital can be used for induction of drug metabolism. In cattle, the enzyme induction mechanisms of phenobarbital have been suggested for use in speeding the detoxification of insecticide poisoning (Plumb, 1999). With the administration of phenobarbital, there is a marked proliferation of the smooth endoplasmic reticulum, which can be correlated with an increase in microsomal enzymes (Conney, 1967). Some of the most important oxidative drug and steroid metabolizing enzymes that are responsive to phenobarbital induction are several of the cytochrome P450 monooxygenase isozymes of the endoplasmic reticulum. The induction of activities of these enzymes by phenobarbital was shown to involve the transcriptional activation of the expression of their respective genes, thus increasing mRNA levels (Omiecinski et al., 1985).

Pentobarbital is a short acting barbiturate metabolized by microsomal drug enzymes primarily in the liver. Barbiturate-induced anesthesia is a popular model of pharmacological or toxicological response, because it is a non-destructive measure of liver function (Lovell, 1986 a, b). Pentobarbital is the drug of choice for this procedure. Length of sleeping time after anesthesia is inversely related to the rate of drug metabolism. Among the many genetic and environmental variables that may affect sleeping time in laboratory mice are age, sex, strain, litter, type of bedding, painful stimuli, and time of drug administration (Lovell, 1986 a, b). Pentobarbital sleeping time is an *in vivo* indicator of drug metabolism, which can be carried out on large numbers of animals over a short interval of time.

Genetic Variation in Toxicosis Resistance

Ruminants. Lipsey et al. (1992) ranked Angus bull calves according to the increase in their body temperature in response to simultaneous heat and fescue toxicosis stress. When subjected to heat stress alone, progeny of the highest ranking and lowest ranking sire did not differ in body temperature response. However, body temperatures did differ between progeny groups when calves were subjected simultaneously to fescue toxicosis and heat stress, suggesting a heritable component to fescue toxicosis resistance. Thus selection for resistance to fescue toxicosis may help to alleviate the problem.

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Gould and Hohenboken (1993) compared progeny of a Polled Hereford bull reputed to have produced calves resistant to fescue toxicosis to those of a Polled Hereford bull with unknown resistance. The calves were tested sequentially on diets containing or not containing E+ fescue seed. The two progeny groups did not differ in most measured physiological responses to fescue toxicosis. However, the progeny of the bull reputed to produce resistant calves had lower body temperatures and higher voluntary feed intake, both in the presence and the absence of E+ fescue. Apparent resistance may therefore have been caused by differences in thermal biology and/or appetite rather than actual resistance to toxins in E+ fescue.

Brown et al. (1997) grazed Angus, Brahman, and reciprocal crossbred cows mated to Polled Hereford bulls on common bermudagrass or E+ fescue pastures. While purebred Brahman cows had similar conception rates and calf production per cow exposed to mating on both pasture types, conception rate and calf production of Angus cows were reduced by 14% and 48%, respectively, on fescue compared to bermudagrass pastures. Therefore, variation among breeds may play a part in resistance to fescue toxicosis.

Sporodesmin is found in the spores of the ryegrass fungus *Pithomyces chartarum* and causes facial eczema, a disease common in New Zealand cattle and sheep. Genetic differences in susceptibility to sporidesmin toxicosis have been reported. Differences found among sheep breeds in susceptibility to sporidesmin have been correlated with pentobarbitone sleeping time (Smith et al., 1980). Merinos were significantly more resistant to sporidesmin poisoning and had significantly shorter pentobarbitone sleep time, suggesting that their greater resistance to sporidesmin intoxication might be due to a more active hepatic microsomal drug metabolizing system. Campbell et al. (1975, as cited by Morris et al., 1989) reported large variation among sheep in susceptibility to facial eczema as determined by Phase II enzyme gamma-glutamyltransferase (GGT) activity (an indicator of liver damage). The heritability of facial eczema susceptibility was estimated to be 0.42. Morris et al. (1989) selected rams for increased resistance to facial eczema and reported a response of 2.4% more resistant animals per year over a five year span. Following 12 years of divergent selection in sheep, the resistant line had much lower incidence of the disease than the susceptible line (Morris et al., 1989). Following 21 years of selection, resistant line individuals, after a disease challenge, had lower GGT concentrations than their susceptible line counterparts (Morris et al., 1991). Field incidence of the disease and resultant financial losses have been substantially reduced in New Zealand flocks using rams performance tested for disease resistance using a toxin challenge developed in conjunction with this research. Selection for resistance to facial eczema has also been effective in dairy cattle (Morris et al., 1991) in New Zealand. Sporidesmin metabolism involves destruction of a disulfide bridge. Genetic differences in susceptibility may reflect differences in the rate of degradation of the disulfide bridge (Briggs et al., 1994).

Morris et al. (1995) reported a correlation of 0.31 between breeding values for resistance to ryegrass staggers and breeding values for resistance to facial eczema, suggesting a common biochemical pathway may be utilized by the sheep to detoxify the compounds. Ryegrass staggers is a neurotoxic disease caused by ingestion of endophyte-infected perennial ryegrass containing the mycotoxin, lolitrem B. It causes muscular incoordination in animals and in sheep is most obvious when they are under stress. The

effects of the toxin are reversible, in that symptoms are alleviated when the stress and the toxin are removed. The selection response indicates that it should be possible to change the degree of ryegrass staggers susceptibility in sheep by selection using progeny-testing under field conditions. Research has not been done to determine correlations between susceptibilities to ryegrass staggers and fescue toxicosis.

Mice. Although laboratory rodents have previously been used as a model to study fescue toxicosis, there does not seem to be evidence other than in Virginia Tech experiments to be described in which selection has been exerted to alter resistance. Godfrey et al. (1994) reported that a non-selected control mouse line and a line that had undergone long-term selection for increased litter size did not differ quantitatively in toxin-induced growth rate depression of lactating females and their pups to an E+ fescue containing diet.

In mice, eight generations of bi-directional selection successfully produced both endophyte resistant and susceptible lines as measured by growth rate response to the toxin in the diet (Hohenboken and Blodgett, 1997). The toxin-containing diet had a greater detrimental impact on long-term reproduction of susceptible line than resistant line mated pairs, and mature weight of susceptible mice was more severely depressed by the toxin-containing diet than was mature weight of the resistant mice (Wagner et al., 2000). Resistant line mice had higher activities of glutathione-S-transferase and uridine diphosphate glucuronosyl-transferase liver enzymes than mice of the susceptible line, both in mice that had and had not received the toxin-containing diet (Hohenboken and Blodgett, 1997). The function of these enzymes is to conjugate foreign, often toxic molecules with endogenous compounds to create complexes more easily excreted in the urine (deBethizy and Hayes, 1994). Biochemical pathways involving these enzyme families seem to be at least partially responsible for line differences in toxicosis response, but there may be other important biological differences as well.

Gavora and Spencer (1978) suggested that selection for disease resistance of a general nature would be more desirable and practical than selection for resistance to specific disease. This may have occurred in the Virginia Tech divergent selection experiment. Hohenboken et al. (2000) reported that divergent selection for resistance and susceptibility to fescue toxicosis resulted in parallel divergence in susceptibility to the mycotoxin, sporidesmin, as well. It would be interesting to see if this population of mice is also resistant to the mycotoxin, lolitrem B, the causative agent of ryegrass staggers in sheep.

Selection. Even though animals differ genetically in their abilities to cope with the challenge of fescue toxicosis (Lipsey et al., 1992; Gould and Hohenboken, 1993; Brown et al., 1997), heritable variation among cattle in resistance to fescue toxicosis may be slow, difficult and expensive to identify. Genetic selection would take years to accomplish and for it to work at all, careful planning and long term breeding objectives must be established for choosing heritable and measurable traits (Gibson and Wilton, 1998).

Future selection criteria may include liver detoxification ability (Hohenboken and Blodgett, 1997), decreased rectal temperature (Lipsey et al., 1992), adequate average daily gain under toxic conditions (Brown et al., 1997), a lower reduction in prolactin levels (Porter et al., 1995), molecular markers, or immune modifications (Gavora and Spencer, 1978). Effective selection would require that these traits be highly correlated with tolerance to fescue toxicosis, vary between animals, and have adequate heritability. Many of the physiological traits that respond to fescue toxicosis may have too much extraneous variation to be reliable indicators of inherent resistance of individuals.

Bourdon (1998) believes that if genetic evaluation is to be effective, it should be broadened to include multiple-trait selection technology that focuses on economic selection indexes. Livestock improvement in the corporate livestock industry has been accomplished by focusing on the economics of production through the use of these selection indexes, which provide a basis for organization and direction of positive

contributions of the breeding population. Economic selection indexes have provided meaningful direction, consistent selection, and a basis for corporate or breed structure. This may prove difficult in cattle because of the diversity of production systems which presents complications such as genotype x genotype, genotype x environment, and other complex interactions.

Materials and Methods

Animals and Management

Mice used in these experiments were from lines divergently selected for resistance (R) and susceptibility (S) to fescue toxicosis as described by Hohenboken and Blodgett (1997). Foundation animals were from an outbred ICR population (Harlan Sprague Dawley, Inc., Indianapolis, IN). During eight generations of bi-directional selection, mice were evaluated for toxicosis response by the reduction in two-week gain when fed a toxin-containing diet (E+), compared to the previous two-week gain when their diet (E-) was free of the toxin. The selection criterion was an index combining information from the animal's own phenotype and that of its littermates.

Throughout the current experiments, mice were housed in a single room of the university Laboratory Animal Vivarium. Temperature was maintained at an average of 24ºC and fluorescent lights were on from 0700 to 1900 each day. Mice were housed in 15 cm x 21 cm x 29 cm transparent plastic cages bedded with a paper fiber product and equipped with automatic watering system lexits.

Experiment 1

A total of 240 male mice, 120 per line, representing 31 S litters and 33 R litters were used in the experiement. Mice were weaned on a single day when litters were between 21 and 26 d of age. Mice were randomly assigned four per cage with the provision that cage-mates were of the same line but were not siblings.

Five dietary treatments, as follows, were assigned to animals in each line immediately following weaning:

1. Control. Finely ground laboratory rodent food (Teklad 70-01, Harlan Sprague Dawley, Madison, WI.)
2. E+. Ground and thoroughly mixed diet composed by weight of one half rodent

food (as above) and one half endophyte-infected KY-31 variety fescue seed.

3. E+P. Same as diet 2 but supplemented with 1000 ppm of phenobarbital.
4. E-. Ground and thoroughly mixed diet composed by weight of one half rodent food (as above) and one half endophyte-free Forager variety fescue seed.
5. E-P. Same as diet 4 but supplemented with 1000 ppm of phenobarbital.

Phenobarbital was added to diets 3 and 5 to induce hepatic Phase 1 enzymes of the cytochrome P450 class (Smith and Reynard, 1992). There were 6 cages (24 mice) per line x diet subclass. Mice were weighed individually at weaning (the start of the trial) and after 1, 2, 3, and 4 wk on the experimental diets. Mice were provided ad libitum access to diets, and fresh food was provided three times per week.

All 240 mice were then administered sodium pentobarbital anesthesia, half of the cages of each line x diet combination (120 mice) on each of two consecutive mornings. Following the protocol of Lovell (1986 a, b), each mouse was anesthetized by i.p. injection of sodium pentobarbital at 40 mg/kg of weight. The anesthetic was dissolved in physiological saline and administered 0.1 mL per 10 g of body weight. Clock time was recorded at injection and when the mouse could no longer accomplish the righting reflex. The mouse was then placed on its back in a standard cage. Clock time was again recorded when the righting response was regained, defined as the ability of the mouse to right itself two times within 30 s. Sleep latency was the elapsed time between injection and loss of the righting reflex, and elapsed time between losing and regaining the righting reflex was called sleep time.

Following the second day of sleep testing, all mice were switched to a diet of pelleted rodent food (Teklad 70-01, Harlan Sprague Dawley, Madison WI). A second weight and sleep time test from sodium pentobarbital anesthesia took place 1 wk later on one fourth of the population (60 mice), consisting of one randomly chosen mouse from each cage. Following that trial, these males were killed by CO_2 asphyxiation. One week later, a weight was taken and a sleep time test was administered on an additional 60 mice, one randomly chosen from each cage. This continued for two more weeks decreasing the

number of mice per cage by one each week until each mouse had been tested twice, with intervals between first and second test being 1, 2, 3, or 4 wk.

Statistical Analyses

Prior to analysis of variance, the variable sleep time was subjected to logarithmic transformation in order to more nearly normalize the distribution of observations and to reduce heterogeneity of variance. Results from statistical analyses were similar for sleep time and its logarithmic transformation. Consequently only the sleep time results will be presented and discussed.

The mathematical model for analysis of variance of the first sleep latency and sleep time for all mice included fixed effects for line, diet, and their interaction, the source of variation of greatest interest. The model was ST_1= _ + L + T +(LxT) + error, where where ST_1= sleep time 1 (when the entire population underwent anesthesia), _= the mean, L=line, and T=dietary treatment. All analyses of variance utilized GLM procedures is SAS (1999).

Cumulative weight gain (CW) of individual mice after 1,2,3, and 4 wk on the experimental diets was analyzed using the model: CW1, CW2, CW3, CW4 = _ + L + T +(LxT) + error.

Two questions were relevant from data collected on the second sleep time of the mice. First, did line and diet effects on sleep time persist after the mice had been switched to a standard lab rodent diet for various lengths of time? To answer this question, all of the sleep time 2 data (ST_2=second time the mice underwent anesthesia) were subjected to ANOVA with the mathematical model ST_2= _ + L + T + D + (LxT) +(LxD) + (TxD) + error, where D=date of second test and other factors are as defined above.

The second question was, what is the relationship between a mouse's first and second sleep time observation, and does this relationship change as time between the first and second measurement increases? To answer this question, data for ST_1 and ST_2 were analyzed simultaneously according to the model $ST_{1,}\ ST_2 = \_ + L + T + (LxT) + error$, and the residual correlation was computed between ST_1 and ST_2. This analysis was done separately for groups whose second sleep time test followed the first by 1, 2, 3, and 4 weeks. The relationship between ST_1 and ST_2, pooled across times between tests, also was quantified as the linear regression coefficient of ST_2 on ST_1 from the model $ST_2 = \_ + L + T + D + (LxT) + (LxD) + (TxD) + \_(ST_1 - \overline{ST}_1) + error$, where _ is the regression coefficient of ST_2 on ST_1.

Experiment 2

A total of 280 individuals, 140 per line**,** representing 35 S litters and 34 R litters were used in the experiment. Mice were weaned on a single day when litters were between 21 and 26 d of age, and mice were randomly assigned four individuals per cage, with the provisions that cage-mates were of the same line and sex but were not siblings. The diet that was administered to all mice for the first two weeks following weaning was the same as E- in Experiment 1. All mice were then challenged for two weeks with the E+ diet from Experiment 1. Mice were then put on a control diet of standard pelleted laboratory rodent food (Teklad 70-01, Harlan Sprague Dawley, Madison WI) for two weeks. At six weeks, all mice (280) were administered sodium pentobarbital anesthesia, one third of the cages of each line x sex combination on three consecutive mornings using the protocol used for sleep time testing in Experiment 1.

Mice were weighed individually at weaning and then weekly for the duration of the experiment. Weight gains were computed for each of the diet periods, E-, E+, and pelleted rodent food. Toxicosis response of each individual mouse was quantified as the reduction in their growth rate when E+ fescue seed was present in the diet, compared to

their growth rate with E- composed the same diet proportion. Cumulative weight gains were computed for the 6-wk duration of the experiment.

Statistical Analyses

As in Experiment 1, results from statistical analyses for sleep time and its logarithmic transformation were similar. Consequently only the sleep time results will be presented and discussed.

Experiment 2 sought to determine whether there were line differences in sleep time following sodium pentobarbital anesthesia, as had been found in Experiment 1 under a different dietary regimen. The model for analysis of variance of sleep latency and sleep time included fixed effects for line, sex, and the line x sex interaction, ST= _ + L + S + (LxS) + error.

Experiment 2 also was designed to see if sleep time following sodium pentobarbital anesthesia of individual animals was correlated with the impact of E+ fescue in the diet on their post-weaning growth (the trait originally used as the criterion for selection for toxicosis response). To answer this question, we computed the residual correlation between ST and R_M from data analyzed according to the above model, where R_M is the percentage reduction in gain associated with E+ feeding. We also analyzed the effect of line, sex, and their interaction on E-GN, E+GN, and SGN, where E-GN=mouse gain since weaning while on the E- treatment; E+GN=gain of mouse while on the E+ diet from the last day of E- to the last day of E+; and SGN=mouse gain from the last day of the E+ diet to the last day of the pelleted rodent food diet.

Results

Experiment 1

Non-responders. Nineteen of 240 mice (Table 1) were not completely anesthetized by the standard dose of sodium pentobarbital and thus had no sleep time measurement. This included ten resistant mice and nine susceptible mice. Although chi-square testing did not detect significant differences in proportion of non-responders among lines or diets, 12 of 19 non-responders had been on phenobarbital-containing diets.

Table 1. Numbers of mice not completely anesthetized per subclass of 24 individuals.

Line	C	E+	E+P	E-	E-P
R	2	3	2	1	2
S	1	0	4	0	4

Sleep Latency. Mean sleep latency was 5.6 min with a standard deviation of 4.9 min. As shown in Table 2, this trait did not differ significantly between R and S mice, nor among dietary treatments. The interaction also was not significant.

Table 2. Mean squares and P-values from analysis of variance of sleep latency and sleep time 1.

	Sleep Latency		Sleep Time 1	
	Mean Square	**P value**	**Mean Square**	**P value**
Line	1.9	.78	1294.8	<.01
Diet	19.3	.52	3731.7	<.01
Line x Diet	24.6	.40	34.7	.94
Residual	24.0	---	172.9	---

Sleep Time 1. For sleep time 1 (ST1), line and dietary treatment main effects were statistically significant, but the interaction was not (Table 2). Least squares means for main effects are shown in Table 3, and line x diet subclass means are shown in Figure 2.

Table 3. Least squares means (± standard error) for line and dietary treatment effects on sleep time following sodium pentabarbitol anesthesia.

Line	**N**	**Sleep time 1 LS means ± SE**
R	110	20.9 ± 1.3^{a}
S	111	25.8 ± 1.3^{b}
Dietary Treatment		
C	45	25.6 ± 2.0^{b}
E+	45	$29.3 \pm 2.0^{b,c}$
E+P	42	13.7 ± 2.0^{a}
E-	47	34.2 ± 1.9^{c}
E-P	42	13.7 ± 2.1^{a}

*Means with different superscripts differ significantly (P<.05).

Susceptible mice slept longer than resistant mice overall and on each of the dietary treatments. While there wasn't a significant difference in sleep time between the E+ and E- diets or between the E+P and E-P diets, there was a significant difference in the sleep time between those mice that were fed the phenobarbital inducer and those that were fed other diets (Table 3). Differences in sleep time between dietary treatments were similar in both lines of mice.

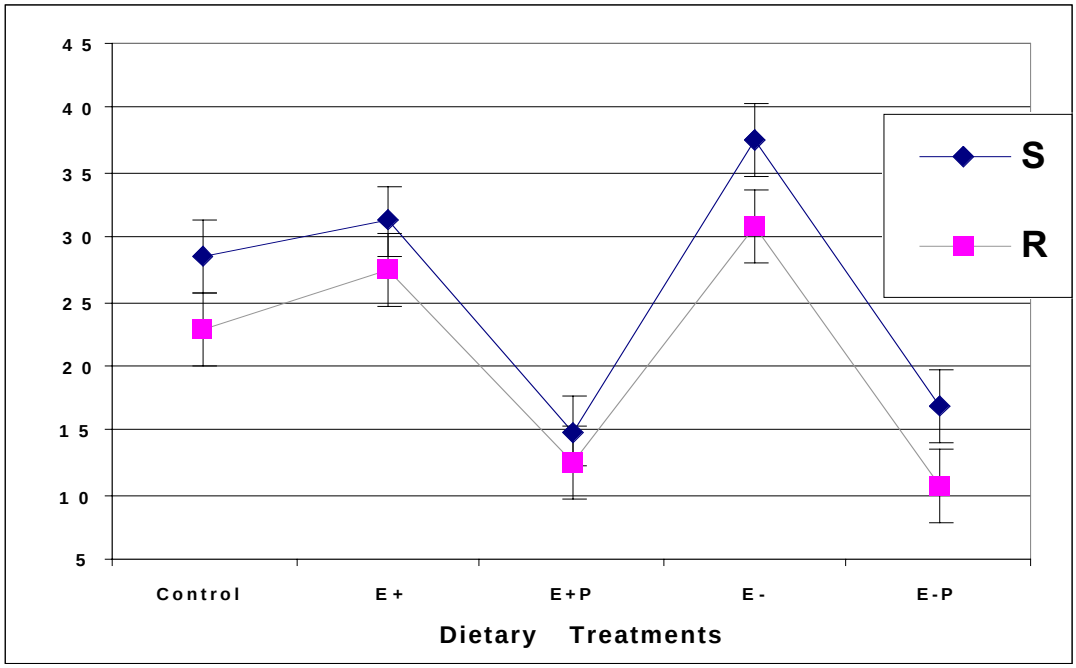

Figure 2. Least squares mean sleep time for line by dietary treatment groups (brackets illustrate plus and minus one standard error for each mean.)

Cumulative Weight Gain. As shown in Table 4, lines did not differ significantly for cumulative weight gain, and there was no line x diet interaction. The dietary treatment effect became significant after wk 1. As shown in Figure 3, mice on the control diet had the highest cumulative gain. Mice on the E- diet gained more than those on the E+ diet. Mice on both the phenobarbital-containing diets had increased gains over those on the E+ diet, which had the poorest gain. The addition of phenobarbital in the E- diet had little effect on growth until after the third week, whereas in the E+ diet the phenobarbital had a small apparent beneficial influence after the first week.

Table 4. Mean squares and P-values from analysis of variance of cumulative weight gains while on the experimental diets.

	CW1		CW2		CW3		CW4	
	Mean Square	**P value**	**Mean Square**	**P value**	**Mean Square**	**P value**	**Mean Square**	**P value**
Line	0.3	.61	6.4	.10	16.5	.09	8.5	.25
Diet	0.9	.58	17.6	<.01	47.5	<.01	65.8	<.01
Line x Diet	1.5	.33	2.1	.46	8.1	.23	11.6	.12
Residual	1.3	---	2.3	---	5.7	---	6.3	---

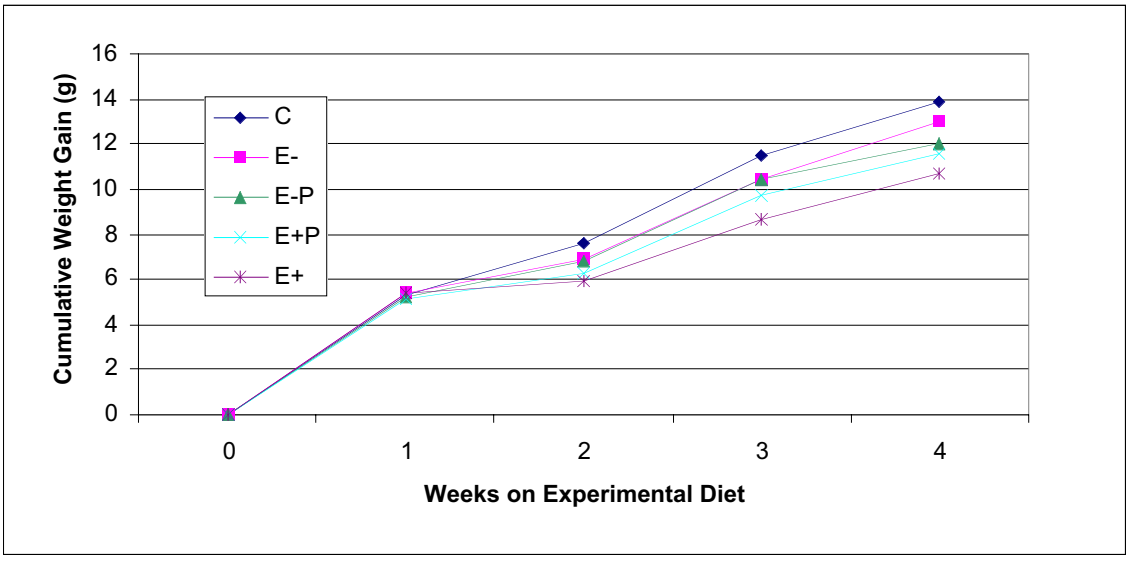

Figure 3. Cumulative weight gain during 4 weeks on experimental diets. Week 0 is weaning. Standard errors of diet subclass means were 0.17, 0.23, 0.36, and 0.38 for wk 1-4 respectively.

Sleep Time 2. In the second part of the experiment, we sought to determine whether line and previous diet effects on sleep time persisted after mice had been on a standard laboratory rodent food diet from 1 to 4 wk. As shown in Table 5, the results of the first analysis found that only line was significant. As shown in Table 6, S mice slept on average, almost eight minutes longer than R mice for ST_2, compared to a 5-minute longer sleep time for ST_1. Although dietary treatment effects were not statistically significant, groups supplemented with phenobarbital had longer ST_2 than groups on the E- or the E+ diet (Table 6). For ST_1, the phenobarbital-supplemented groups had much shorter sleep times than these other two groups. Time interval between ST_1 and ST_2 had no significant impact on the duration of sleep time.

Table 5. Mean squares and P-values from analysis of variance of sleep time 2.

	Sleep Time 2	
	Mean Square	**P value**
Line	3168.3	<.01
Diet	304.1	.28
Line x Diet	282.6	.31
Date	198.1	.47
Line x Date	537.7	.08
Diet x Date	225.5	.49
Residual	235.6	---

Table 6. Least squares means (± standard error) for line, dietary treatment, and time interval effects on the second sleep time measurements on the mice.

Line	N	Sleep Time 2
R	109	21.2 ± 1.5^{a}
S	104	29.0 ± 1.5^{b}
Dietary Treatment		
C	44	27.5 ± 2.3
E+	42	21.5 ± 2.4
E+P	41	25.5 ± 2.4
E-	45	23.4 ± 2.3
E-P	41	27.8 ± 2.4
Time Interval (wk)		
1	52	22.7 ± 2.2
2	55	26.9 ± 2.1
3	52	26.6 ± 2.1
4	54	24.4 ± 2.1

Means with different superscripts differ significantly at P<.01.

A second analysis sought to quantify the relationship between a mouse's first and second sleep time and asked whether this relationship changed as the time between the first and second measurement increased. Although mice with above average ST_1 tended also to be above average for ST_2, the correlations were only moderate (Table 7). Although not significant, as time between measurements increased and the mice aged, there was a trend for residual correlations to decrease. When the first sleep time was fit as a covariate, the regression was 0.44 ± 0.08. Thus, for every 1 minute deviation (positive or negative) from average for ST_1, that same mouse was expected to deviate (positive or negative) 0.44 minutes from average in ST_2.

Table 7. Residual correlations between sleep time 1 and sleep time 2 for mice whose second sleep time followed their first by varying intervals of time.

Time Interval (wk)	Residual Correlation
1	0.43
2	0.40
3	0.31
4	0.29

Experiment 2

Non-responders. Sixteen of 280 mice did not have a valid sleep time measurement. Eight of these were because of injection or observational errors and eight were not completely anesthetized by the standard sodium pentobarbital dose. Because the number of non-responders was small, a chi-square analysis was not performed to test for line or sex differences.

Sleep Latency. Mean sleep latency was 4.7 min with a standard deviation of 1.8 min. As shown in Table 8, this trait was not influenced by line, sex, or their interaction.

Table 8. Mean squares and P-values from analysis of variance of sleep latency and sleep time.

	Sleep Latency		Sleep Time	
	Mean Square	**P value**	**Mean Square**	**P value**
Line	7.9	.13	1080.7	<.01
Sex	0.9	.61	260.7	.15
Line x Sex	0.2	.80	70.4	.45
Residual	3.4	---	125.2	---

Sleep Time. As shown in Table 8, sleep time was significantly influenced by line but not by sex or the interaction. As in Experiment 1, susceptible mice slept longer than their resistant counterparts (Table 9).

Table 9. LS means and standard errors for main effects on sleep time.

Line	N	LS means ± SE
R	134	22.3 ± 1.0
S	130	26.3 ± 1.0
Sex		
F	134	23.3 ± 0.97
M	130	25.3 ± 0.98

Relationship of Sleep Time to Fescue Toxicosis Susceptibility. There was no overall relationship between reduction in gain associated with the E+ diet (R_M) and sleep time, as indicated by the residual correlation of 0.04 between these two variables. From this correlation, we conclude that knowledge of a mouse's sleep time is not a reliable indicator of its weight gain response to fescue toxicosis.

Tables 10 and 11 show that females had a higher percentage reduction in gain associated with E+ feeding than did males. However there was no significant difference between lines as might have been expected.

Table 10. Mean squares and P-values from analysis of R_M, E-GN, E+GN, and SGN.

	R_M		E-GN		E+GN		SGN	
	Mean Square	**P value**	**Mean Square**	**P value**	**Mean Square**	**P value**	**Mean Square**	**P value**
Line	406.0	.39	2.9	.36	17.9	<.01	11.8	.04
Sex	22383.2	<.01	646.3	<.01	397.2	<.01	14.5	.02
Line x Sex	107.8	.66	0.3	.78	12.0	<.01	0.9	.56
Residual	548.4	---	3.5	---	1.5	---	2.8	---

Table 11. LS means and standard errors for R_M.

Line	LS means ± SE
R	71.3 ± 2.0
S	68.8 ± 2.1
Sex	
F	79.3 ± 2.0
M	60.8 ± 2.1
Line x Sex	
RF	79.9 ± 2.9
RM	62.7 ± 2.9
SF	78.7 ± 2.9
SM	59.0 ± 3.0

Weight Gain. During the first two weeks of the experiment, when all mice were on the E- diet, males grew significantly faster than females but R and S mice grew at a similar rate (Figure 4). The line x sex interaction effect was not significant. In the following two week period, while mice were on the E+ diet, line, sex, and the line x sex interaction all were significant sources of variation. In combination with advancing age of the animals, the toxic diet appears to have caused a depression in growth rate. The males grew faster than the females with the S males growing the fastest. R and S females grew at the same rate during this period. In the final two week period, when mice were fed pelleted rodent food, line and sex effects were significant, but the interaction was not. The growth rate increase in the females may have been due to compensatory gain. The males grew at a similar rate between 2-4 wk and 4-6 wk.

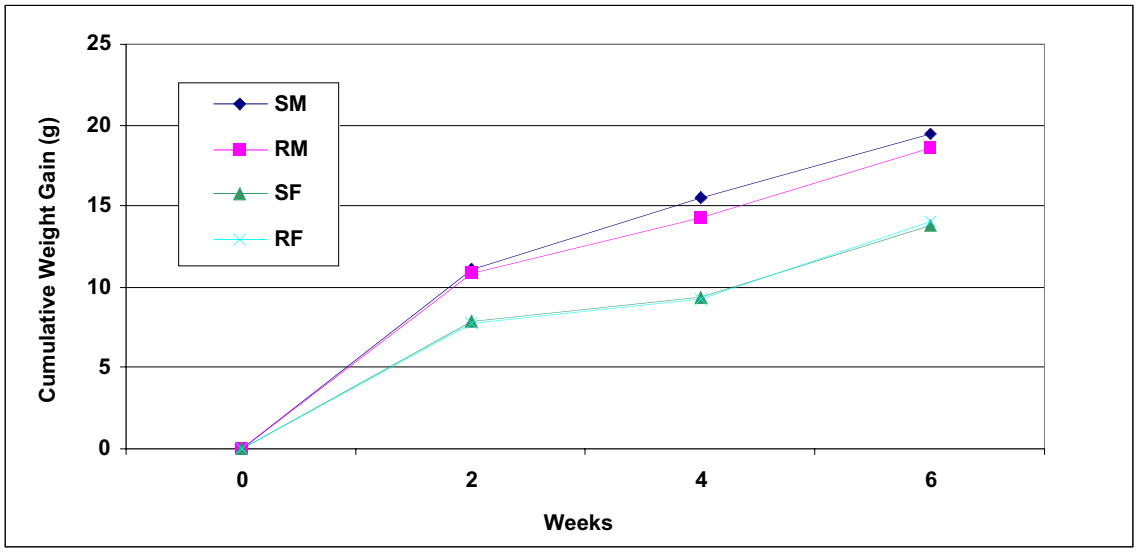

Figure 4. Mouse growth during Experiment 2. Wk 0 is weaning. Wk 0-2=E-, Wk 2-4=E+. Wk4-6=Standard laboratory rodent food. Standard errors were 0.23, 0.15, and 0.20 for wk 2, 4, and 6 respectively.

Discussion

R and S Mice Differ in ST. As might have been expected in both of these experiments, S mice slept longer than R mice. This suggests that R mice had an increased concentration of cytochrome P450, increased hepatic protein synthesis, and/or an increased rate of biotransformation, which hastened the clearance of sodium pentobarbital from the circulation. The consistent difference in sleep times in the experiments suggests that a difference exists between lines in Phase I enzyme activity. Previously only monofunctional induction (induction of Phase II enzymes without induction of Phase I enzymes) has been reported in this population of mice. Hohenboken and Blodgett (1997) found that there was an increase in activity of the Phase II biotransformation enzymes, glutathione-S-transferase and uridine diphosphate glucuronosyl-transferase, in the resistant line of mice. However, they were unable to confirm differences in Phase I enzyme activity by measuring liver cytochromes P450 and b5, two enzymes that have been reported to be induced by more than 200 large lipophilic xenobiotics (deBethizy and Hayes, 1994). In that experiment mice were weaned and then fed for 2 wk on the E- diet followed by either 4 wk on the E+ diet or an additional 4 wk on the E- diet. Neither of the two Phase I enzyme activities was significantly affected by selection line, diet, or their interaction. Future work should directly compare different Phase I enzyme activities in the two lines.

Lovell (1986 a) confirmed genetic influences on pentobarbitone sleep time by finding a highly significant difference between two inbred mouse lines. In subsequent experiments (Lovell, 1986 b), various environmental influences affected the two lines similarly, but line differences in sleep time were reversed from the earlier experiment, suggesting the existence of a genotype x environment interaction. A strong linear correlation between breed group means for pentobarbitone sleep times and sporidesmin resistance was reported by Smith et al. (1980). They theorized that Merino sheep had greater resistance to sporidesmin intoxication due to a more active hepatic microsomal drug metabolizing system (as suggested by shorter sleep times). Subsequent studies

established that microsomal fractions of Merino livers were more efficient at metabolizing sporidesmin than microsomal fractions of Romney sheep livers and that cytochrome P450 content of microsomes was greater in Merinos than in Romneys.

Line Differences in Sleep Time Were Not Modulated by Diet, Phenobarbital Induction, or Time. In Experiment 1, line and diet significantly influenced ST_1, but the line x diet interaction effect, the source of variation of greatest interest, was not significant. That is, the difference in ST between the R and S line was very similar across the five dietary treatments. In particular, the addition of phenobarbital to the diet (a known Phase I enzyme inducer) neither increased nor decreased the difference in sleep time between lines. Likewise, the difference was consistent across the control, E-, and E+ diets. Susceptible line mice presumably did take longer to clear pentobarbital from circulation, but the response pattern and differences in response values were similar to those of the R mice with each treatment. Across diets, we might have expected there to be variable differences or different patterns in the induction response between the R and S lines knowing that Hohenboken and Blodgett (1997) had reported differences in Phase II enzyme activities.

Under a different dietary regimen in Experiment 2, line differences in sleep time following sodium pentobarbital anesthesia were similar to results found in Experiment 1. S mice again slept longer, suggesting a delayed or reduced metabolic response, compared to the R line.

Results from the analysis of the second sleep time in Experiment 1 demonstrate that line differences in sleep time persisted but that dietary treatment effects did not, once the mice were returned to a standard laboratory rodent food diet. Pooled across mice whose second sleep time followed their first by 1, 2, 3, and 4 wk, the linear regression of ST_2 on ST_1, was 0.44 ± 0.08. Thus, on their second test, mice tended to retain nearly half of any deviation from average sleep time on their first test. From examination of residual correlations between ST_1 and ST_2, there was a non-significant trend for the

correspondence between ST_1 and ST_2 to decrease with advancing time on the uniform diet and advancing mouse age. As ST is an indicator of Phase I activity on liver metabolism, this mechanism may help us to understand the reasons for reversibility in conditions in some toxicoses such as fescue toxicosis and rye grass staggers, in that symptoms are alleviated when the stress and the toxin are removed.

Differences Among Individuals in ST Were Not Correlated with Differences Among Individuals in Fescue Toxicosis Response. Based on the residual correlation of 0.04, there was virtually no relationship between sleep time following sodium pentobarbital anesthesia of individual animals and the impact of E+ fescue in the diet on their post-weaning growth (the trait originally used as the indicator of toxicosis response during the selection phase of the research program). Divergent selection had been successful in differentiating the lines for weight gain response to the E+ diet (Hohenboken and Blodgett, 1997) and (as shown in these experiments) for sleep time following sodium pentobarbital anesthesia. Consequently mice that had a higher reduction in gain might have been expected to sleep longer; and animals with an active metabolism, that slept the shortest time, may have been expected to be more resistant. However, individuals within lines with greater than average weight gain response to toxicosis did not deviate from average in sleep time. Similarly, Smith et al. (1980) were not able to show a within-breed correlation between pentobarbitone sleep time and sporidesmin resistance in sheep.

This may be due to noise (error variance) in the reduction in weight gain measurement. That is, weight gain reduction may reflect individual toxicosis resistance with poor accuracy. Selection may have been successful in creating these lines because an individual and family selection index was used (Hohenboken and Blodgett, 1997), which increases accuracy of selection, particularly for lowly heritable traits.

Weight Gain was Depressed by E+ Fescue in the Diet. In Experiment 1, diet did not affect gain during the first week; but during the next three weeks, there were significant differences due to dietary treatments. Control mice had the highest cumulative gain, followed by those on the E- diet. These E- mice may have been impacted by ergot alkaloids other than those known to be produced by the E+ fescue seed. Alternatively, the 50% fescue seed, 50% rodent food diet may have been nutritionally inadequate or aversive to the taste. The next highest gain was on E-P and E+P diets respectively. Phenobarbital induction caused a similar sleep time reduction, and by inference a similar accelerated clearance of pentobarbital on both E+ and E- diets in both lines of mice. However, phenobarbital seems to have enhanced gain in mice on the E+ but not on the E- diet. Mice fed E+ without phenobarbital had the lowest cumulative gain of all groups.

Conclusions. Based upon these and previous studies, divergent selection for toxicosis response in mice was successful partially by causing divergence between lines both in Phase I and Phase II liver detoxification enzyme activities. Ultimately, genetic selection will not provide the complete solution to fescue toxicosis in cattle, but if a heritable, practical, and economical criterion could be identified to quantify such differences in livestock species, then selection for toxicosis resistance may contribute to the solution of this important problem for American agriculture.

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Implications

Hepatic microsomal drug metabolism (biotransformation) is a complex multipathway system. Unrelated detoxifying pathways may coexist and influence sodium pentobarbital sleep times or the fate of toxins from E+ fescue and may contribute to the greater resistance and shorter sleep times of the R line. Additional possible resistance mechanisms include differences in thermoregulatory biology or preabsorptive degradation of the toxins. Irrespective of the mechanism responsible, these results raise the possibility that resistant aspects of a genotype, if found in cattle, may be utilized in selection for fescue toxicosis resistance.

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Vita

Kimberly Ann Arthur, daughter of Warren and Joyce Arthur, was born in Fort Riley, Kansas. She grew up in Berryville, VA and attended Clarke County High School. Following high school she attended James Madison University in Harrisonburg, VA where she graduated with a B.B.A. in Business Marketing. Following an education "hiatus" in Colorado, she returned to Virginia to pursue a Master of Science degree in Breeding and Genetics from the Department of Animal and Poultry Sciences at Virginia Polytechnic Institute and State University in Blacksburg under the direction of Dr. William D. Hohenboken. She successfully defended her thesis in January 2002. While completing her research Kimberly matriculated at the Virginia-Maryland Regional College of Veterinary Medicine and will receive her Doctor of Veterinary Medicine degree in May 2003.

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Kimberly Ann Arthur