THE EFFECT OF THREE FESCUE TYPES AND LAKOTA PRAIRIE GRASS ON COPPER STATUS, DRY MATTER INTAKE, AND ALKALOID APPEARANCE OF BEEF STEERS

Robert Lawton Stewart, Jr

Dissertation submitted to the faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

In
Animal and Poultry Sciences
(Ruminant Nutrition)

Guillermo Scaglia, Chair
Azenegashe O. Abaye
John H. Fike
Joseph P. Fontenot
Mark A. McCann
William S. Swecker, Jr.
Eric A. Wong

October 27, 2006
Blacksburg, VA

Keywords: Tall Fescue, Beef Steers, Copper, Intake, Alkaloids

THE EFFECT OF THREE FESCUE TYPES AND LAKOTA PRAIRIE GRASS ON COPPER STATUS, DRY MATTER INTAKE, AND ALKALOID APPEARANCE OF BEEF STEERS

Robert Lawton Stewart, Jr

ABSTRACT

Tall fescue [*Schedonorus arundinaceus* (Schreb.) Dumort.] is an important forage crop in the United States and covers over 14 million ha. The presence of *Neotyphodium coenophialum*, an endophytic fungus in tall fescue, is associated with several disorders in grazing livestock, but also increased persistence of tall fescue. These disorders, commonly called fescue toxicosis, are responsible for large economic losses in the beef cattle industry each year. This research examined the effect of three fescue types [endophyte-infected Kentucky 31 tall fescue (E+), endophyte-free Kentucky 31 tall fescue (E-), non-ergot alkaloid-producing endophyte Q4508-AR542 tall fescue (Q)], and Lakota prairie grass (L; *Bromus catharticus* Vahl.) on animal response, alkaloid appearance, DMI, and copper status.

Ergovaline (EV) is the most abundant ergot alkaloid in tall fescue and has previously been considered the causative toxin in fescue toxicosis. More recently it is simpler ergot alkaloids, such as lysergic acid amide (LSA) have been implicated. The objective of the first project was to evaluate animal performance and alkaloid (EV and LSA) appearance in forage and ruminal fluid of steers grazing E-, Q, E+, and L. Average daily gains were greater ($P < 0.05$) on E-, Q and L compared to E+, and there was a trend ($P = 0.11$) for gains on E- to be higher than with Q. The seasonal appearance of LSA in ruminal fluid was similar to the seasonal pattern of alkaloids in E+ forage. Ergovaline was not detectable in ruminal fluid of steers grazing E+. Alkaloids were not detectable in

forage or ruminal fluid of steers grazing E-, Q, or L. The appearance of LSA in ruminal fluid of steers grazing E+ suggests that this alkaloid may contribute to fescue toxicosis.

Low DMI of animals grazing E+ tall fescue is considered a key factor in decreased animal performance compared to other fescue types. The objective of the second project was to evaluate DMI of steers grazing E-, E+, Q, and L pastures using the alkane technique. Dry matter intake of steers grazing E- was greater ($P < 0.001$) than Q, E+, and L and DMI of steers grazing Q and E+ were similar ($P > 0.10$) in 2004. In 2005, DMI did not differ ($P = 0.23$) among fescue types. These results suggest that decreased DMI effects ADG of steers grazing E+ compared to those grazing E-, and lower DMI of Q suggests that the fescue variety Q4508 may not be the optimal variety for the incorporation on non-ergot alkaloid-producing endophytes.

Reactive oxygen metabolites such as superoxide (O_2^-) are produced by both endogenous and exogenous sources and an accumulation of these compounds can result in oxidative stress. Copper/zinc superoxide dismutase (SOD) is a Cu-based antioxidant metalloprotein that acts as a defense against oxidative stress by the scavenging of O_2^-. *Neotyphodium*-infected tall fescue is typically lower in Cu which could potentially increase oxidative stress of animals grazing this forage. Therefore the objective of the third project was to investigate the Cu and SOD status of steers grazing E-, E+, Q, and L forages. Copper levels of all forages were below the dietary requirement (10µg Cu/g DM) of growing cattle. In 2004, steers grazing E+ exhibited lower ($P <0.05$) liver Cu compared to E- and Cu intake was lower ($P < 0.001$). Cu/Zn SOD enzymatic activity and mRNA relative expression did not differ ($P > 0.10$) among treatments. Copper

intake of steers grazing E+ tall fescue was sufficient to maintain, but not replenish liver Cu, and SOD status did not appear compromised by grazing E+ at these Cu levels.

To my wife, Beth.

ACKNOWLEDGMENTS

The past three years (and two months) have been an amazing journey that would not have been possible without the help of many people. I would like to begin by thanking Dr. Guillermo Scaglia, chair of my committee, for his guidance and expertise in the field of Ruminant Nutrition, and his patience. I would like to show appreciation to my committee, Dr. Mark McCann, Dr. William Swecker, Dr. Ozzie Abaye, Dr. Joseph Fontenot, Dr. John Fike, and Dr. Eric Wong, for your willingness to serve on this committee, thoughtful advice, and review of this dissertation. Also, I express my thanks to the John Lee Pratt foundation for its financial support of my graduate program.

This graduate program would not have been possible without the collaboration and guidance of other scientists and the use of their labs. This includes Dr. George Rottinghaus at the Veterinary Diagnostic Lab, College of Veterinary Medicine, University of Missouri; Dr. Eugene Gregory, Biochemistry Department, Virginia Tech; and Dr. Eric Wong, Animal and Poultry Sciences Department, Virginia Tech.

Thanks are due to the many hands that helped me along the way both in the field and lab. This includes Tina Shanklin for her guidance in the lab, willingness to put in long hours in the field, and friendship along the way. Also, thanks to fellow Ruminant Nutrition graduate students (in no particular order) Carrie Pickworth, Alexis Lillie and Holly Boland. These ladies assisted with countless hours of data collection in the field, and were great friends along the way. The help of Gary Bradley and Henry Dickerson with working cattle is greatly appreciated.

Also, thanks to the many friends I have made through this experience. There are too many to mention by name, but my graduate career is full of fond memories outside of academics that I will take with me, and for these, I am thankful.

Last, but not least, I would like to thank my family. My wife, Beth, has been supportive of my decision to pursue a Ph.D. and the many tasks that go along with it. I believe she is the only Speech Language Pathologist capable of the proper 'sampling' techniques involved with dry matter intake estimation. Also I would like to express my gratitude to my parents, Robert and Martha Stewart. They have always emphasized the importance of education and supported me throughout my many journeys in life. To my sisters Kate and Sally, who have encouraged me, pestered me, but more importantly been my friends for as long as I can remember, thank you. Also, thanks are due to my in-laws, the Haleys, for their support along the way.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1
INTRODUCTION

Tall fescue (*Lolium arundinacea* Schreb.) is widely utilized throughout the eastern United States as a forage resource for beef cattle production systems due to its persistence, versatility, and acceptable nutritive value. The presence of the fungal endophyte *Neotyphodium coenophialum* provides the positive agronomic qualities of fescue partially through the production of alkaloids. While the mutualist relationship with this fungus is beneficial to the plant, fungal alkaloids are responsible for the deleterious effects on animals grazing tall fescue including fescue foot and fat necrosis in severe cases, and more commonly fescue toxicosis. These conditions have been estimated to affect over 8.5 million cattle (Rumball and Miller, 2003) and previously estimated to cost the beef cattle industry $609 million (Hoveland, 1993).

The first release of fescue as a forage crop occurred in the 1942 as 'Kentucky 31'. It grew in popularity and was widely planted due to both its persistence under many environmental and management conditions and acceptable nutritive value. However, , producers soon began to notice health issues associated with the grazing fescue (Conrad et al., 1964). Initially, researchers were not aware of the early work of Neill (1940) investigating endophytes in fescue, and the endophyte was rediscovered in the 1970s (Bacon et al., 1977). Since this discovery, attempts have been made to alleviate the toxic effects of tall fescue. These, among others, include the removal of the endophyte from the seed (Hoveland et al., 1982). This resulted in increased animal performance, but the persistency associated with tall fescue was severely decreased and stand survival was

reduced (Bouton et al., 2002). Bacon and Seigel (1988) first proposed that the fungal endophyte might be modified to produce only the beneficial agronomic properties of fescue while eliminating the toxic effect on animals. This was first made possible with the discovery of non-toxic endophyte strains in New Zealand (Latch et al., 2000). This allowed the elimination of the alkaloids associated with fescue toxicosis while maintaining the positive characteristics of tall fescue.

Once the endophyte was identified, several alkaloids were isolated from endophyte infected plants including ergot alkaloids (Bacon and Siegel, 1988). This class of alkaloids was targeted as the toxicosis-causing agents, and due to ergovaline's high levels in plant material, it was specifically pinpointed as the causative alkaloid (Agee and Hill, 1994). Hill et al. (2001) indicated that transport of ergovaline across ruminal gastric tissue was low compared to the smaller alkaloids, lysergic acid and lysergol. Also, alkaloid appearance in urine occurs relatively quickly, within 48 h, and urinary excretion patterns were similar to alkaloid solubility patterns from in vitro digestion of E+ tall fescue (Stuedemann et al., 1998). Due to the rapid liberation from plant material and appearance in urine, the excretory route for smaller (less than 350 Da) alkaloids, these authors suggested smaller alkaloids such as lysergic acid amides and/or biotransformed ergopeptines could be the toxic element of fescue.

The beef cattle industry is a crucial component of the Virginia economy comprising of 1.54 million heads and providing $339 million in cash receipts to the state's economy in 2003, second only to broiler production (VDACS, 2005). Virginia is twentieth in the nation in total number of cattle and fourth east of the Mississippi River. Fescue is a vital

component of Virginia agriculture covering approximately 0.5 million ha, (Vazquez and Smith, 2000) and it is utilized predominantly by the beef cattle industry.

Although alternative forages to endophyte-infected (E+) tall fescue exist, including endophyte-free (E-) and non-toxic fescues, the cost associated with replacing E+ tall fescue can be high. Conversion costs associated range from $125-620/ha depending on method used (Burdine and Trimble, 2005). At an average of $370/ha, cow-calf producers would have to increase weaning weights by approximately 25 kg and stocker operators would need to increase production 0.25 kg/d over a 10-yr period to produce positive returns (Burdine and Trimble, 2005). In addition to economic costs, agronomic traits associated with the endophyte infection may be jeopardized, especially when replacing E+ with E- tall fescue. Therefore, if the physiological pathways within the animal are better understood, measures could be taken to continue utilizing this excellent forage, while alleviating the deleterious effects related to fescue toxicosis.

With these facts in mind, the present work is divided into three research areas. The first study investigated the effect of E+, E-, non-ergot alkaloid-producing endophyte-infected tall fescue(Q) and Lakota prairie grass (L; *Bromus catharticus* Vahl.) on animal response measured as average daily gain and diurnal tympanic temperature. Additionally, forage and ruminal fluid appearance of the ergot alkaloids ergovaline and lysergic acid amide were quantified. The second research study investigated the DMI of steers grazing E-, E-, Q, and L using alkanes as markers. Intake estimates for grazing animals has proven difficult but the use of markers, both internal and external, can aid in this determination. Little literature is available regarding use of alkanes to determine diurnal differences in DMI of fescues and Lakota prairie grass. This study also compared

DMI estimation using different sampling methods: hand plucked samples to mimic the animal's diet as well as whole plant samples. The third study the Cu status, gene expression and enzyme activity of the Cu-based enzyme Cu/Zn-superoxide dismutase (SOD) in steers. Copper levels have shown to be lower in E+ tall fescue (Dennis et al., 1998), and Cu is an essential component of SOD, a free radical scavenger. Decreased SOD activity could possibly contribute to the toxicity of E+ tall fescue. A decrease in SOD would lead to an increase in free O_2^- radicals, increasing oxidative stress.

The aim of this research was not to solve the current problem producers face with fescue toxicosis. This research potentially increases our current understanding of fescue toxicosis and comparison of animal response on E+ tall fescue to alternative forages. These results may lead to future strategies to understand the physiological paths of fescue toxicosis, and in turn, improve performance of animals grazing E+ tall fescue.

CHAPTER 2
LITERATURE REVIEW

TALL FESCUE

Tall fescue [*Schedonorus arundinaceus* (Schreb.) Dumort.] is a deep-rooted, cool-season perennial grass. Although it produces short rhizomes, tall fescue has a bunch type growth habit, spreading primarily by erect tillers (Duble, 2004). Tall fescue provides strong growth in the spring and fall. Under proper management, the grass is deep rooted and forms a dense sod resistant to drought, but will also tolerate wet soils and brief flooding. Each tiller terminates in an inflorescence in spring reaching 90 to 120 cm in height with seed maturing in early summer. The basal leaves are broad, dark green, and leaf blades are serrated on the margins with glossy undersides. The ligule is a short membrane and the leaf sheath is smooth. The inflorescence is a compact panicle, three to four inches in length with spikelets approximately 2 to 3 cm long (Craven et al., 2005).

Tall fescue is native to Europe. Time of its introduction to the United States is not exactly known, but had been tested in several places by the late 1800s (Buckner et al., 1979). The grass was of little importance until the rediscovery by Dr. E.N. Fergus (University of Kentucky). In early winter, 1931, while visiting a farm in eastern Kentucky, Fergus found a grass, still green being grazed by cattle. It covered the hill side, protecting it from erosion. Fergus obtained seed for subsequent research and the grass was released as 'Kentucky 31' tall fescue in 1942 (Lacefield and Evans, 1984). Kentucky 31 was widely accepted and planted by producers throughout Kentucky and

eventually spread throughout much of the United States, now covering over 14 million ha (Thompson et al., 2001).

Although tall fescue proved to be an excellent forage based on its agronomic characteristics, producers noticed an ill thrift of animals grazing it especially during the summer months. This ill thrift associated with tall fescue is now referred to as fescue foot, fat necrosis, and more commonly summer slump or fescue toxicosis (Rumball and Miller, 2003). In the 1950s, research objectives began to focus on determining the cause of deleterious effects. In the 1970s Bacon et al. (1977) were the first to note the association of a fungus with the incidence of animal health issues and isolated the fungus, hypothesized to be *Epichloe typhina*, from two varieties and three hybrids of tall fescue. *Epichloe typhina* is an endophytic fungus which causes a disease in grasses known as "choke" where the mycelium of the fungus emerges and forms a weft around the developing inflorescence preventing seed development. However, tall fescue never exhibited outward signs of a fungal infection. It was determined that the endophytic fungus infecting tall fescue was *Neotyphodium coenophialum*, an asexual fungi related to *E. typhina* (Latch, 1997).

PRAIRIE GRASS

'Lakota' prairie grass (*Bromus catharticus* Vahl.) is a relatively new bromegrass to the United States (Rumball and Miller, 2003). Lakota is a cool-season, short-lived perennial bunch grass with erect growth, typically 80 to 100 cm in height. The leaves of Lakota are light green to green with an open, drooping panicle inflorescence with flat spikelets (Abaye et al., 2002). Little literature is currently available on this cultivar of prairie grass, but it is similar to 'Matua' prairie grass (*Bromus willdenowii* Kunth). Lakota is superior to Matua as a forage crop because it is less susceptible to powdery

mildew [*Blumeria graminis* (DC) Speer] due to its more open and wider crown. It also has a less erect structure and does not head as early as Matua (Rumball and Miller, 2003). Prairie grasses in general are adapted to cooler temperatures than other forages. These forages are high in nutritive value, palatable and endophyte free, but require longer periods of rest between grazing period and does not persist under continuous grazing due to their intolerance to frequent, intense defoliation (Abaye et al., 2002). Unlike most cool-season grasses, it produces seedheads over the entire growing season and by allowing seedheads to form, stand persistence can be increased (Jung et al., 1994). Matua has been described as an excellent forage for producers to extend the grazing season because it is adapted to growing earlier in the spring and later in the fall than most cool season grasses (Abaye et al., 2002). The negative aspect of prairie grass is its lack of grazing tolerance and needs a longer rest period between grazing sessions to ensure stand persistence.

Abaye et al. (2002) evaluated herbage mass of Matua prairie grass and tall fescue over two years in three locations in Virginia. Herbage mass of prairie grass in Blacksburg, VA was greater than tall fescue (16 vs 13.5 ton/ha) in the first year with similar production between forages in year two (approximately 20 ton/ha). Herbage mass was greater in Blacksburg compared to other Virginia locations due to lower temperatures and higher rainfall.

LaCasha et al. (1999) evaluated voluntary intake and digestibility of Matua, 'Coastal' bermudagrass (*Cynodon dactylon* L.) and alfalfa hays as feed for horses. Voluntary DMI was greatest ($P < 0.01$) for alfalfa (10.9 kg/d) and DMI of Matua was greater ($P < 0.001$) than that of bermudagrass (10.0 vs 7.4 kg/d, respectively). Apparent

OM digestibility was similar between Matua (64 %) and bermudagrass (60 %), but both were lower ($P < 0.001$) than alfalfa (74 %).

Lowe et al. (1999)evaluated herbage mass, persistence and nutritive value of Matua prairie grass, tall fescue, and perennial and Italian ryegrasses for lactating Holstein-Friesian cows grazing these forages. Over three years, average herbage mass for both Matua and tall fescue (2191 and 1980 kg DM/ha, respectively) was higher ($P < 0.05$) than Italian and perennial rygrasses (1641 and 1567 kg DM/ha, respectively). Matua, Italian and perennial ryegrass had higher ($P < 0.05$) weed encroachment (477, 539, and 535 kg DM/ha, respectively) compared to tall fescue (98 kg DM/ha). Milk production was highest for cows grazing perennial ryegrass and Matua (16.7 and 16.2 kg/d), respectively), intermediate for cows grazing Italian ryegrass (15.9 kg/d), and least for cows on tall fescue (15.2 kg/d). Milk production was similar across forages in yr 2. In yr 3 Matua supported the highest milk production (22.6 kg/d); $P < 0.05$) compared to Italian ryegrass (21.0 kg/d) with fescue and perennial ryegrass being intermediate (22.4 and 22.4 kg/d).

ENDOPHYTE FUNGAL INFECTION

Neotyphodium coenophialum is an asexual fungus of the family Clavicipitaceae that shares a mutualistic relationship with tall fescue (White, 1994). The endophyte spends its entire lifecycle within the plant and, therefore, infected fescue shows no outward signs of fungal infection. The fungus is found in the intercellular spaces as coarse, mostly unbranched, linear or contorted hyphae, which run vertically between the host cells (Siegel et al., 1987). During vegetative and dormant stages, the fungus is located in the meristematic tissue of the shoot apex. In the spring, when the plant is initiating flowering shoots, the endophytic mycelia are found primarily within the

intercellular spaces of the pith cell. *Neotyphodium* endophytes are anamorphic or imperfect fungi, that do not reproduce sexually (Clay, 1988). The only mode of propagation of the endophyte is through the seed. Within the seed, the fungus is located between the scutellum of the embryo and the aleuron layer (Faeth et al., 2004). The endophyte enhances growth characteristics of the plant, increases resistance to different stresses and provides a defense against herbivores (Schardl et al., 2002). In return, the endophyte receives a protected environment in which to live, nutrients, and a means by which to propagate asexually. This increased persistence is provided, in part, by the production of alkaloid compounds, which act to deter other organisms from feeding on tall fescue (Schardl et al., 2002).

Alkaloids

Alkaloids are chemicals with one or more nitrogen atoms bound to carbon, hydrogen, or combinations of carbon and hydrogen atoms. The term alkaloids actually refers to the alkaline nature of these compounds (Porter, 1995). Alkaloids isolated from fescue which are associated with the fungus *N. coenophialum* can be classified into three categories based on structure: pyrrolizidine (loline), ergot alkaloids, and the pyrrolopyrazine alkaloid, peramine. Peramine has been associated with insect resistance and has not been shown to contribute to endophyte toxicity (Porter, 1995).

Loline alkaloids (Figure 2-1) have a multi-cyclic structure comprised of two 5-membered rings sharing adjacent carbon and nitrogen atoms, plus an oxygen atom bridging those rings (Blankenship et al., 2001). Two major forms of loline alkaloids have been isolated from tall fescue: N-acetyl and N-formyl loline. Concentrations of loline alkaloids increase with plant age, foliage regrowth, and N fertilization, and are found in foliage at concentrations up to 0.1-1.0 % of dry weight (Larson et al., 1999). The role of

these compounds in fescue toxicosis has been debated, and has been suggested to have synergistic effects with the ergot alkaloids in fescue toxicosis (Siegel et al., 1987). Studies have shown their activity as D2 dopamine agonists to be minor (Strickland et al., 1994; Larson et al., 1999), and lolines (N-formyl, N-acetyl, and N-methyl) showed no effect on prolactin secretion in rats' isolated anterior pituitary (Strickland et al., 1992). From these studies it is apparent that the loline alkaloids have little effect on grazing livestock. However, these compounds have shown to be highly toxic to a wide range of insects, and have been implicated in drought tolerance. (Siegel et al., 1987; Larson et al., 1999; Wilkinson et al., 2000).

Chemically, ergot alkaloids are 3,4-substituted indol derivatives having a tetracyclic ergoline ring structure (Figure 2-2). Ergot alkaloids are categorized into two broad classes, ergoline alkaloids and ergopeptine alkaloids. Both classes contain the tetracyclic ring structure, but ergoline alkaloids contain a carboxyl or carboxamide functional group at the 8-position, where ergopeptine alkaloids have a tripeptide cyclol moiety attached to the carboxyamide site (Tudzynski et al., 2001).

Previous studies have pointed to ergovaline as the toxic alkaloid causing fescue toxicosis due to its high recovery from endophyte-infected (E+) tall fescue (Agee and Hill, 1994). Westendorf et al. (1993) fed increasing amounts of E+ feed to abomasally cannulated sheep to measure the abomasal and fecal appearance of alkaloids. These data showed that 47 to 63% of ergot alkaloids were present in abomasal digesta, but only 6% was present in the feces. From this, the authors suggested that ergot alkaloid absorption occurred post-ruminally. Stuedemann et al. (1998) studied ergot alkaloid excretion through urinary and biliary routes. Supernatant alkaloid as a percent of total alkaloids

present in the rumen increased from 19% at 0 h to 58% at 48 h showing the solubility of alkaloids in rumen fluid. The majority of the alkaloids (94%) were excreted in the urine, and the rest (6%) in bile. In addition, the rate of appearance and clearance of these alkaloids in steers grazing E+ or endophyte free (E-) tall fescue was measured. In steers switched from E- to E+ tall fescue, urinary alkaloid levels were similar to those of animals continuously grazing E+ tall fescue within 2 d ($P = 0.55$). Steers moved from E+ to E- fescue had similar urinary alkaloid levels after 2 d ($P = 0.91$). Due to the speed of appearance in urine and solubility in rumen fluid Stuedemann et al. (1998) suggested that alkaloid absorption occurs anterior to the duodenum.

The microbial population present in the rumen is able to metabolize ergopeptine alkaloids into simpler alkaloids such as ergoline alkaloids by peptide cleavage or proline transformation (Eckert et al., 1978). From this, it is likely a mixture of alkaloids is present in the rumen. Hill et al. (2001) investigated the in vitro relative and potential transport of alkaloids (ergoline and ergopeptine) across isolated gastric tissue of sheep. Compared to omasal tissue, ruminal tissue had a greater ($P < 0.05$) potential for alkaloid transport (60 vs 85 mmol, respectively) and transport of the ergoline alkaloids lysergic acid and lysergol (42 and 25 mmol, respectively) was greater than ergopeptide alkaloids (6 mmol). Due to the site and higher potential for transport, these authors suggested that a simple ergoline alkaloid is responsible for fescue toxicosis contrary to the previous belief that ergovaline (an ergopeptine alkaloid) is responsible for this condition. From this previous research, it is apparent that the exact cause of fescue toxicosis is still unknown.

Altering the plant-endophyte relationship

Once fescue toxicosis was associated with the fungal endophyte *N. coenophialum* and the alkaloid compounds it produced, efforts were made to alleviate the toxicity by removing the fungus. Storage of E+ seed under ambient temperature and humidity generally results in the death of the endophyte within a year (Hoveland, 2003). The first endophyte-free (E-) fescue cultivar, 'AU Triumph' was released in the early 1980s. Experiments conducted with this cultivar showed higher animal ADG compared to E+ tall fescue (Hoveland et al., 1982). However it was quickly noted that pastures of E- tall fescue lacked the drought, insect, and grazing tolerance of E+ tall fescue. In addition, E+ tall fescue crowns are located deeper in the soil compared to crowns of E- tall fescue (Hill et al., 1990). Therefore, without additional management, E- tall fescue types lack the overall stand persistency compared with E+.

There is considerable evidence that some species of grass, including tall fescue, require infection by their respective endophytes for maximal survival under stressful environmental conditions (Clay, 1988). As mentioned earlier, loline and peramine alkaloids are associated with insect and drought resistance, but there is no strong evidence linking them to mammalian toxicoses. With this in mind, efforts have been made to develop E+ cultivars with reduced or no production of toxic (ergot) alkaloids (Bouton et al., 2002). The objectives would be to reduce alkaloid levels through genetic selection of the plant genotype-endemic strain association (Adcock et al., 1997), identify and isolate genes vital to alkaloid production in E+ endophytes and genetically manipulate these strains for future infection (Tsai et al., 1995), or select for naturally occurring, non-toxic lines of the fungus for reinfection into selected cultivars (Latch et al., 2000).

Previous studies have shown that ergot alkaloid concentrations vary depending on tall fescue genotype (Agee and Hill, 1994; Roylance et al., 1994), implying a plant-endophyte interaction in the production of these compounds. Alkaloids are produced by the endophyte; however, plant genotype may mediate this production through calcium and tryptophan nutrition provided to the endophyte (Roylance et al., 1994). Both of these components are crucial in the biosynthesis of ergot alkaloids (discussed below). Calcium concentration in tall fescue is variable, and this trait is highly heritable (Sleper, 1979). Increased calcium concentration in tall fescue increases ergot alkaloid production (Roylance et al., 1994). Due to the effect of plant genotype on ergot alkaloid production, there is potential to develop cultivars with lower alkaloid production through selection. This, however, does not eliminate the alkaloids from the plant and therefore does not eliminate the toxicosis problem.

The pathway of ergot alkaloid biosynthesis by *N. coenophialum* (Figure 2-3) was determined by Schardl et al. (2002). Tryptophan, isoprene (in the form of dimethylallyl diphoshate) and methionine (methyl group donor) are the precursors for the lysergic acid moiety of the alkaloids. The first determinant and rate-limiting step of ergot alkaloid production by the endophyte is the prenylation of tryptophan to 4-(γ, γ-dimethylallyl) tryptophan (DMAT), catalyzed by dimethylallyl-diphosphate:L-tryptophan dimethylallyltransferase (DMATase), and Ca is a coenzyme for DMATase (Panaccione and Schardl, 2003). Genes coding for enzymes involved in these pathways have begun to be cloned from the endophytes. The first gene cloned was *dmaW*, which is responsible for DMATase, the first step in the pathway (Tsai et al., 1995). Later, the gene *lpsA* responsible for lysergic peptide synthetase was identified and its role in the biosynthesis

of ergovaline has been demonstrated by gene knockout (Panaccione et al., 2001). This peptide synthetase is part of the complex that converts lysergic acid and three amino acids (alanine, phenylalanine, and proline) to an ergopeptine lactam. After this step, the final cyclization step (gene identified) converts this lactam to the ergopeptine structures commonly found in tall fescue. Isolation of these genes from the endophyte associated with tall fescue could possibly lead to the development of genetically modified strains of *N. coenophialum* and provide another avenue of eliminating ergot alkaloid production from tall fescue.

The most successful attempt to reduce alkaloid production in tall fescue has come from the selection of naturally occurring non-toxic lines of endophyte. Currently the development of these cultivars has centered around two research groups: the Georgia, USA—New Zealand group (Drs. Bouton and Latch) and the Arkansas—Missouri group (Drs. West and Sleper).

Several strains of endophytes incapable of producing ergot alkaloids, including: AR501, AR502, AR510, AR542, AR572, AR577, and AR584 have been developed (Latch et al., 2000). Reinfection of two tall fescue cultivars, Jesup and Georgia 5, with AR542 improved animal performance of grazing animals and stand survival compared to wild-type E+ (Bouton et al., 2002). Jesup and Gerogia 5 AR542 plots planted in South Georgia in March 1997 had stand coverage of 17.0 and 23.5 % in January of 1999. This was statistically higher ($P < 0.05$) than the E- types of these cultivars (2.0 and 2.3 %, respectively), and equivalent to the E+ cultivars with wild-type endophytes (22.3 and 36.6 %, respectively). In central Georgia, overall stand survival was greater across all types, but similar differences between types were observed. Stand survival of Jesup

AR542 (38.5%) was similar to survival with wild endophytes (47.5%) and survival of both were greater ($P < 0.05$) than Jesup E- types (10.7%). Stand survival of Georgia 5 with AR542 endophyte was less ($P < 0.05$) than with wild types (24.7 vs. 91.7%), but greater ($P < 0.05$) than E- plants (9.5%). Dry matter production was greater ($P < 0.05$) for Jesup 542 (9.1 Mg/ha) compared to E- types (7.8 Mg/ha) with E+ types (8.6 Mg/ha) having intermediate yields. Dry matter production was similar ($P < 0.05$) across all Georgia 5 types.

The West-Sleper group selected endophytes that were ergopeptine deficient (West and Prohaska, 2004). These endophytes were incorporated into 'HiMag' fescue, an E- cultivar with a low risk of causing grass tetany in cows (Sleper et al., 2002), to produce HiMag4 and HiMag9.

FESCUE TOXICOSIS

Fescue toxicosis is the general term that indicates a negative impact of consuming infected tall fescue. Animal responses to tall fescue endophytic toxins associated with fescue toxicosis can be grouped into four categories (Stuedemann and Thompson, 1993): (i) animal performance defined as decreased weight gain and pregnancy rate, (ii) behavioral conditions demonstrated with decreased dry matter intake and increase water intake, (iii) physiological responses including increased respiration rate and elevated rectal and core body temperatures, and (iv) decreased in levels of plasma constituents such as prolactin and cholesterol. These symptoms are exacerbated by elevated temperatures, thus this condition is more obvious during the summer months.

Animal performance

Decreased animal gains and reproductive rates are the greatest economical impact that endophyte-infected tall fescue has on the beef cattle industry and are linked to and a result of the other categories mentioned.

Several studies have measured performance of animal grazing E+ tall fescue along with other grasses including E- and non-toxic endophyte fescues. Parish et al. (2003) compared average daily gains (ADG) of steers grazing four Kentucky-31/Jesup-5 tall fescue treatments—AR542-infected and AR502-infected (novel endophyte strains), E-, and E+ at two locations in Georgia (central and northern) over 3 yr. There was no cultivar by endophyte status interaction. Treatment x season interactions were reported at the central Georgia location because ADG of calves on E+ fescue were lower ($P < 0.05$) in spring compared to fall (0.31 vs 0.56 kg/d). The authors attributed this to the presence of seedheads. In the spring, tall fescue enters a reproductive stage in which alkaloids concentrate in the seedheads (Rottinghaus et al., 1991). Calf ADG was higher ($P < 0.05$) on the AR542, AR502, and E- fescues when compared to E+ fescue in the spring (0.95, 0.78, 0.96, and 0.31 kg/d, respectively) and in the fall (0.81, 1.00, 0.87, and 0.56 kg/d, respectively). Cattle grazing the same type of pastures in northern GA showed comparable differences ($P < 0.05$) between forages, gaining 0.73, 0.72, 0.71, and 0.49 kg/d in spring and 0.82, 0.82, 0.83, and 0.41 kg/d in fall (AR542, AR502, E-, and E+ tall fescues, respectively).

Nihsen et al. (2004) evaluated animal performance of steers grazing four tall fescue types including two with non-ergot alkaloid-producing endophytes (HiMag4 and HiMag9), E+ Kentucky 31, and E- HiMag fescues, over a 2 yr period in northern Arkansas and southern Missouri. Average daily gains of animals grazing E+ fescue were

significantly lower ($P < 0.05$) than those grazing both HiMag4 and HiMag9 and E- (0.34, 0.60., 0.54, and 0.61 kg/d, respectively). In addition, steers grazing E+ fescue had elevated ($P < 0.05$) respiration rate (107 vs. mean of 82 breaths/min), and rectal temperatures (41.1 vs. mean of 40.1 ºC).

In a study conducted in Tennessee over three springs and two fall/winters (Waller et al., 2001) compared animal performance of steers grazing Kentucky-with no endophyte or wild or AR542 strains. In the fall/winter, cattle grazing E+ and AR542 fescues had similar gains (0.99 and 0.97 kg/d, respectively), but both were significantly lower ($P < 0.05$) than steers grazing E-(1.60 kg/ha). In the spring, cattle grazing E+ tall fescue gained significantly less ($P < 0.05$) than those grazing E- and AR542 (1.06, 1.64, and 1.62 kg/ha, respectively).

Bouton et al. (2002) reported that over three grazing seasons, ADG of lambs grazing Jesup E+ were lower ($P < 0.05$) than those grazing Jesup with E-, AR542 and AR502 strains (78.5, 123.0, 130.3, and 114.9 g/d, respectively).

Alkaloids also reduce circulating hormones and other plasma constituents important in the reproduction cycle of cattle. Browning et al. (1998) investigated the effects of the injecting the ergot alkaloids ergotamine (EM) and ergonovine tartrate (ET) on plasma concentrations of hormones essential to reproductive function in cattle. These constituents included prolactin, luteinizing hormone (LH), follicle stimulating hormone (FSH) and the $PGF_{2\alpha}$ metabolite 13,14-dihydro-15-keto-protaglandin $F_{2\alpha}$ (PGFM). Ergotamine decreased ($P < 0.001$) circulating prolactin one and two hours after EM injection, but levels began to increase three and four hours after injection. Prolactin decreased ($P < 0.001$) 1 h after injection and remained lower than the pre-injection level

through 4 h post-injection. Reduced levels of prolactin have been a well-recorded indication of ergot alkaloid activity and fescue toxicosis in cattle (Porter and Thompson, 1992; Paterson et al., 1995; Samford-Grigsby et al., 1997; Hill et al., 2000; Parish et al., 2003b). Not only lowered prolactin levels serve as a mean of determining fescue toxicosis, but also plays a role in biological functions related to animal reproduction, particularly decreased lactation. Luteinizing hormone, a key regulator of corpus luteum development and function, was not as largely reduced as prolactin by both EM and ET, only being lower ($P < 0.01$) 3 h post-injection for EM and 4 h post-injection for ET. The blocking of $PGF_{2\alpha}$ by the presence of the embryo maintains pregnancy and prevents luteolysis and return to estrus (Bazer, 1992). In control cattle, PGFM after post-injection of saline was not different from zero. Cattle given EM had PGFM levels greater than zero ($P < 0.05$) one and two hours post-injection, but decreased three and four hours post-injection. Cattle given ET increased PGFM above zero ($P < 0.05$) 1 h post-injection and levels remained elevated through 4 h (Browning et al., 1998).

Follicular and luteal development and function in beef heifers is impaired by consumption of endophyte-infected tall fescue and heat stress (Burke et al., 2001). Heifers consuming an E+ diet had reduced ($P < 0.05$) corpus luteum diameter and circulating progesterone when exposed to heat stress (cycle between 25 and 31 °C) compared to heifers in thermoneutral (19 °C) temperatures. The combination of infected seed and heat stress tendended ($P = 0.11$) to reduce diameter of the preovulatory dominant follicle, and the inclusion of E+ tall fescue seed resulted in fewer ($P < 0.04$) large follicles (> 9 mm) during the estrous cycle with no effect of heat stress. These

authors indicate that the presence of heat stress is necessary for the exacerbation of the effects of tall fescue on reproductive function in beef heifers.

Animal behavior and voluntary intake

Animals that graze E+ fescue have also shown changes in behavioral patterns including decreased grazing time, increased lounging time and decreased water intake compared to animals consuming non E+ forages (Howard et al., 1992; Aldrich et al., 1993; Parish et al., 2003b; Scaglia et al., 2005). Howard et al. (1992) reported that the grazing behavior and DMI of steers grazing low endophyte infected Johnson tall fescue and E+ Kentucky 31 tall fescue. Steers grazing E+ tall fescue spent more time standing and idling ($P < 0.10$) and took fewer prehensile bites ($P < 0.05$) during daylight hours compared to those grazing the low endophyte infected tall fescue. Herbage intake did not differ between treatments, but a treatment x period interaction ($P < 0.10$) was observed. Intake of E+ was lower than E- during late May and June when seedheads were present. In free-grazing situations, the effects of E+ tall fescue are not as pronounced as when animals are in controlled environments due to the ability of the animals to select for leaf over stem (Howard et al., 1992). Leaves have lower ergovaline concentrations compared to stem and seedhead (Rottinghaus et al., 1991). Lambs consuming an E+ diet (1,170 ppb of ergovaline supplied by seed) had lower ($P < 0.05$) DMI than lambs consuming an E- diet (1.76 vs. 2.55 % BW). When fed the E+ diet with the dopamine antagonist metoclopramide, intake was similar to that of the E- diet (2.43 % BW). Although not significant, water intake was numerically greater (5.3 vs. 4.2 L/d) for lambs consuming E+ compared to E- (Aldrich et al., 1993). Parish et al. (2003b) evaluated performance and grazing behavior of steers grazing E+, E-, and non-ergot alkaloid-producing endophyte (AR542) infected tall fescues. In central Georgia, cattle grazing E+ pastures

spent more ($P < 0.01$) time idling and standing and consumed more water than cattle on both E- and AR542 fescue during the spring grazing season (April, May, and June). Both biting rate and daily prehensions ($P < 0.01$) were higher on E- and AR542 than E+ fescue in both spring and fall (September, October, and November), but no differences ($P = 0.50$) in bite size were observed. Dry matter intake was lower on E+ pastures compared to both E- and AR542 during the spring ($P < 0.10$) and lower than steers grazing E- pastures during autumn ($P < 0.01$). Water usage was decreased ($P < 0.10$) in late fall on E+ pastures compared to E- and AR542 pastures. This decrease in usage was attributed to ambient temperatures dropping below 32 °C.

Physiological response

Animals that graze E+ fescue have also shown physiological responses including increased body temperature and respiration rates. Increased rectal temperatures of animals grazing E+ tall fescue have been widely reported. Al-Haidary et al. (2001) reported that core temperatures of heifers fed E+ diets were higher compared to E- diets ($P < 0.05$) in a short-term (2-d) constant heat challenge (32°C) study, with the effect most pronounced from 0000 to 0300 and temperatures being similar thereafter. The increase in core temperature was associated with an increase in respiration rate ($P < 0.05$), but no differences in metabolic heat production, skin temperature, skin vaporization or respiratory vaporization were detected. These authors suggested that the heat stress associated with the consumption of E+ diets is due primarily to a reduction in cutaneous heat transfer and not increased heat production or other methods of heat dissipation such as respiratory and skin vaporization. Increased body temperatures have been associated with the animals' inability to dissipate body heat due to vasoconstriction in the peripheral tissue. Isolated bovine lateral saphenous vein (cranial branch) and

dorsal metatarsal artery were treated with lysergic acid amide, an alkaloid present in E+ tall fescue, to investigate its vasoconstrictive properties (Oliver et al., 1993). Bovine vessels showed strong contractile response to lysergic acid amide indicating that this compound, representative of the ergot alkaloids, was responsible for the decreased blood flow to the periphery in animals grazing E+ fescue (Oliver et al., 1993). These authors also showed that isolated lateral saphenous veins from animals grazing E+ pastures had greater contractililty than those grazing E- pastures. Specifically, veins from animals grazing E+ revealed enhanced reactivity only for the α_2-adrenergic receptors (Oliver et al., 1998).

Browning and Leite-Browning (1997) measured the respiration rate, rectal and skin temperature, and systolic and diastolic pressures of steers dosed with either a control (saline), ergotamine tartrate, or ergonovine maleate 15 min prior to and 30, 60, and 90 min after the treatments were administered. Ninety minutes post treatment both alkaloid treatments produced higher ($P < 0.05$) respiration rates in steers compared to the control as well as increased mean arterial blood pressure ($P < 0.02$). Heart rate was lower ($P < 0.02$) for the ergotamine tartrate treated steers compared to the control steers, but ergonovine maleate treated steers were not.

Blood parameters

Research has been conducted to investigate changes in circulating hormones and blood constituents in order to explain the physiological responses of fescue toxicosis. Cattle that graze E+ tall fescue have shown decreased enzymatic activity, particularly hepatic enzymes (Oliver et al., 2000; Waller et al., 2002; Nihsen et al., 2004). Compared to cattle grazing E- tall fescue, cattle grazing E+ tall fescue had greater ($P < 0.001$) albumin to globulin ratio and creatinine and lower ($P < 0.01$) alanine aminotranserfase,

total cholesterol, globulin, and prolactin (Oliver et al., 2000). Values for these metabolites were within normal ranges in both treatments, but lower values in the E+ diet may be due to decreased intake (Oliver et al., 2000). Nihsen et al. (2004) observed that cattle grazing E+ tall fescue had suppressed cholesterol, creatinine, prolactin as well as total triglycerides ($P < 0.05$) compared to cattle on both E- and non-ergot alkaloid-producing endophyte infected tall fescues. Saker et al. (1998) evaluated the immune response of steers grazing E+ and E- tall fescue and determined that animals grazing E+ tall fescue had lower ($P < 0.05$) phagocytic activity and major histocompatibility complex class II expression and suggested that E+ tall fescue compromises the immune function of grazing animals.

Oliver et al. (2000) also evaluated blood cellular parameters of animals grazing E+ and E- tall fescue. Many parameters including hemoglobin, hematocrit, platelets, and white blood cells were not affected by treatment. Erythrocyte numbers actually increased ($P < 0.05$) in steers grazing E+ tall fescue, but erythrocytes were smaller in size (microcytic) and had less hemoglobin content (hypochromic; $P < 0.01$). Oliver et al. (2000) attributed this to reduced Cu levels due to its involvement in hemoglobin synthesis.

Glutathione is a Se-containing enzyme that aides in preventing cellular oxidative damage by reducing free radicals (Kaneko et al., 1997). Lakritz et al. (2002) reported lower ($P < 0.05$) glutathione (3.24 vs 2.42 mmol/L of red blood cells) and higher oxidized glutathione (0.020 vs 0.033 mmol/L of red blood cells) in cattle in thermoneutral conditions (18 °C) compared to cattle consuming an E+ diet under heat stress (alternating 4-h intervals of 26 and 33 °C). Lakritz et al. (2002) attributed these

changes to lower DMI and heat stress on the E+ treatment and suggested that these conditions may induce oxidative stress in cattle.

Molecular mechanism of fescue toxicosis

Over the past 30 yr, many scientific approaches have been utilized to investigate the actual physiological mechanism by which E+ tall fescue causes fescue toxicosis. In recent years scientists have begun to explore molecular avenues of this disorder. The use of microarray analysis is a fairly novel approach to exploring the cellular mechanism. The first report of this method analyzed luteal tissue from heifers fed diets containing E-, E+, or E+ tall fescue supplemented with the dopamine antagonist, domperidone (Suttle and Jones, 1987). These authors found that luteal tissue from heifers in the E+ treatment had 598 genes and expressed sequence tags (ESTs) down regulated and 56 up regulated compared to the E- treatment and fewer comparative differences between E- and E+ with domperidone. The liver is the most important organ concerning xenobiotic metabolism and detoxification, as well as a target organ for fescue toxicosis (Oliver, 2005) and has been the focus of microarray studies (Bhusari et al., 2006; Settivari et al., 2006). Settivari et al. (2006) identified shifts in genetic expression with the physiological response of fescue toxicosis. Core body temperature, feed efficiency, and prolactin were measured as physiological responses, and microarray analysis was used on liver tissue on E- and E+ diets. Rats consuming the E+ diet showed reductions ($P < 0.05$) in mean core temperature, feed intake, feed conversion efficiency, liver weight per unit BW, and serum prolactin. From the microarray analysis, there was downregulation ($P < 0.05$) of genes associated with energy metabolism, antioxidant protection, and growth and development, while genes associated with gluconeogenesis, detoxification, and biotransformation were upregulated. In a similar study using rats as models consuming either E+ or E- diets,

liver tissue was analyzed using DNA microarray (Bhusari et al., 2006). The microarray data identified 36 genes differently expressed between treatments. Genes involved in the sex-steroid metabolism pathway and cholesterol and lipid metabolism were downregulated, while genes coding for ribosome and protein synthesis were upregulated on E+ diets compared to E- diets.

Alleviating fescue toxicosis

Since fescue toxicosis was recognized as a problem in grazing animals, many approaches have been evaluated to reduce or alleviate the effects of E+ tall fescue. These have included both pasture and animal management practices as well as feed and diet additives.

Pasture management

Pasture management practices include replacing E+ tall fescue with E- or non-ergot alkaloid-producing grasses which has been discussed previously, but also practices such as dilution with other grasses and legumes, mowing, stockpiling, and application of compounds to the pasture have been investigated.

Thompson et al. (1993) reported that the incorporation of clover (*Trifolium* spp.) into E+ infected tall fescue increased ($P < 0.05$) ADG of steers by 0.1 kg/d compared to E+ alone. Similarly, Waller and Fribourg (2002) showed ADG of steers grazing E+ tall fescue with clovers was higher ($P < 0.05$) compared to steers grazing E+ fescue alone (0.55 kg/d and 0.44 kg/d, respectively).

In greenhouse conditions, increasing clipping height from 2.5 to 7.5 cm increased concentrations of the alkaloids ergononvine, ergocryptine, perloline methyl ether, and an unknown alkaloid, while ergovaline, ergocristine, and two unknown alkaloids were not affected (Salminen et al., 2003).

Stockpiling is a practice commonly used with tall fescue to provide forage for grazing animals during months of minimal or no herbage growth. In Missouri, Kentucky 31 tall fescue herbage mass did not change from mid-December to mid-March, but ergovaline levels decreased over this time period during both years of the 2-yr study (Kallenbach et al., 2003). The ergovaline concentrations were 454 μg/kg in December of the first year and 175 μg/kg in the second year, but declined by 85% by March of both years. During the first year the rate of loss of alkaloids was greater than the second and the authors suggest that weather affects the process of alkaloid disappearance.

A series of studies were conducted in Virginia and Mississippi to investigate the influence of Tasco™-Forage, an *Ascophyllum nodosum* seaweed-extract product, on animals grazing E+ and E- tall fescues (Fike et al., 2001; Montgomery et al., 2001; Saker et al., 2001; Vallentine, 2001). The application of Tasco to forage did not affect ADG of steers grazing E+ or E- tall fescue (Fike et al., 2001), nor their ADG during the subsequent feedlot period (Vallentine, 2001). In sheep, however, Fike et al. (2001) indicated that animal gains were improved ($P < 0.05$) on E+ pastures by the application of Tasco to forage compared to E+ pastures without Tasco (0.13 vs 0.03 kg/d, respectively). Steers grazing E+ pastures had decreased ($P < 0.05$) monocyte phagocytic acitivty and major histocompatibility class II expression, but this was reversed ($P < 0.05$) by the application of Tasco to pastures. Although there was no effect on steer performance during grazing, Tasco increased marbling score ($P < 0.05$) and improved color stability and extended beef shelf-life ($P < 0.05$) independent of endophyte status (Montgomery et al., 2001; Vallentine, 2001).

Animal management

Different grazing strategies have been studied to determine if fescue toxicosis can be alleviated. These include varying stocking rates and forage availability, and rotational versus continuous stocking. Ergot alkaloid concentrations are highest in the seedhead of E+ tall fescue (Rottinghaus et al., 1991), and thus clipping or intensely grazing during the spring when seedheads are commonly present could decrease the toxicity. Bransby et al.(1988) reported increased ADG and gain per ha for steers grazing E+ tall fescue at heavy stocking rates compared to lighter stocking rate. Gwinn et al. (1998) compared infection rates of E+ pastures managed under low, moderate, and high grazing pressure over 2 yr of grazing. These authors found that under high grazing pressure, infection rates increased by 31 and 38 % when the original endophyte level was 25 and 60 %, respectively, but did not change under high original endophyte level (80%). Under low grazing pressure, the endophyte level was unchanged. Therefore, it was concluded that stress from intense grazing increased the endophyte infestation levels and not environmental stress or pasture contamination (Gwinn et al., 1998).

Although extensive research has been conducted on rotational stocking, little research is available comparing rotational versus continuous stocking or different rotational strategies to alleviate fescue toxicosis. Davenport et al. (1993) evaluated performance of steers grazing E+ and E- tall fescue continuously, and on a 14-d rotation between the two fescue types. There were no differences in ADG or prolactin between animals grazing E+ continuously or the rotational treatment. Coffey et al. (2000) found changing rotation frequency from twice weekly to twice monthly had no substantial differences in cow weights, or actual or adjusted weaning weights.

Feed additives and drugs

Endophyte infected tall fescue has low plant Cu and reduces serum Cu of cattle grazing it. Therefore supplementation with Cu would appear to be a logical practice to overcome this deficiency. Coffey et al. (2000) reported that increased ($P < 0.01$) serum Cu and ceruloplasmin in steers grazing E+ tall fescue supplemented with CuO compared to those without Cu supplementation. However, there was no improvement in steer ADG due to Cu supplementation. Saker et al. (1998) reported that animals receiving CuO bolus in the spring prior to initiation of grazing had an increased ($P < 0.05$) immune response during the month of July on both E- and E+ compared to these treatments without supplementation.

Ammoniation of hay increases nutritive value by increasing CP values. Roberts et al. (2002) reported that ammoniation of E+ tall fescue hay decreased ($P < 0.01$) alkaloid content of forage material compared to green chopped forage, but was not different from non-ammoniated hay. Kallenbach et al. (2006) found ammoniation of E+ straw decreased ($P < 0.05$) ergovaline compared to non-ammoniated (125 vs 183 ppb, respectively). In addition, compared to E+ straw alone, animals fed ammoniated E+ tall fescue increased ($P < 0.05$) both intake (3.7 vs. 4.7 kg/d) and ADG (0.2 vs -0.1 kg/d).

The use of pharmacological agents to alleviate fescue toxicosis has also been investigated. Metoclopramide, a D_2 dopamine receptor blocker, increased ($P < 0.05$) ADG (0.31 vs. 0.15 kg/d) and grazing time (22.4 vs. 6.2% of the time between 1200 and 1600) of steers grazing E+ tall fescue compared to untreated animals (Lipham et al., 1989). When administered to sheep, metoclopramide increased ($P < 0.05$) intake by 27.6% compared to untreated animals, but did not affect core body temperature (Aldrich et al., 1993). Domperidone, another D_2 dopamine receptor blocker, is used primarily in

the equine industry to alleviate fescue toxicosis symptoms in grazing mares (Redmond et al., 1994; Cross et al., 1999). When administered to cycling heifers fed an E+ diet, domperidone increased ($P < 0.05$) ADG compared to heifers fed E+ alone (16.1 vs 9.3 kg) which was not different from animals fed an E- diet (17.0 kg). In addition, blood plasma progesterone increased to levels similar to animals on the E- diet.

Other avenues have been investigated to alleviate fescue toxicosis with conflicting or unsuccessful results. These include feeding activated carbon (Goetsch et al., 1988), aluminosilicates (Chestnut et al., 1991), anthelmintic compounds (Goetsch et al., 1988; Bransby, 1997; Coblentz et al., 2000), and zeranol implants (Goetsch et al., 1988; Brazle and Coffey, 1991).

COPPER

Copper has an atomic number of 29 and an atomic weight of 63.5 and is an essential micronutrient as well as a potential toxin in beef cattle. In ruminants, Cu was first recognized as an essential nutrient in 1931 when grazing cattle in Florida demonstrated wasting disease which was linked to Cu deficiency in addition to Co and Fe deficiency (Becker et al., 1965). Since its discovery as an essential nutrient, Cu has been found to be required for cellular respiration, bone formation, cardiac function, connective tissue development, myelination of the spinal cord, keratinization, and tissue pigmentation. Copper is also necessary for the formation of several physiologically important metalloenzymes (Table 2-1), including superoxide dismutase (SOD), dopamine-β-hydroxylse, cytochrome oxidase, lysyl oxidase, ceruloplasmin, and tyrosinase (Berdanier, 1994). The current National Research Council requirement for all beef cattle is 10 ppm daily(NRC, 2000).

Copper is found throughout many soil types and can range from 10-300 ppm depending on the nature of the soil. However, due to the variety of conditions that affect Cu availability to the plant, soil Cu is not a reliable indicator of plant Cu status. Copper becomes more available in poorly drained, acidic soils, but is reduced by high organic matter and the presence of metallic antagonists such as Mo, S, and Fe (McDowell, 1994). Early research showed liver Cu to decrease from 260 ppm (DM basis) to 44 ppm when cattle were fed diets of 0 and 1600 ppm Fe for 84 d (Standish et al., 1969). These levels of Fe are extreme and are above the maximum threshold of 1000 ppm (NRC, 2000), but do help illustrate the antagonistic effects when present in the diet. The presence of Mo and S in high concentrations leads to the formation of insoluble compounds that are utilized inadequately by the ruminant. Molybdenum and S react to form tetrathiomolybdates. These compounds react with Cu and particulate matter in the rumen resulting in the formation of highly stable compounds which cannot be digested or absorbed (Vallentine, 2001). Therefore, adequate Cu is not only a function of sufficient Cu in the diet, but also the levels of Mo and S. Miltimore and Mason (1971) indicated that in the presence of adequate S, a Cu:Mo ratio of 2:1 in feeds is critical for proper Cu absorption, and a lower ratio results in Cu deficiency. Allway (1973) reported that a Cu:Mo ratio of 4:1 is necessary to ensure the Cu requirement is met. The form in which Cu is fed also affects its absorption in the ruminant animal.

Copper in tall fescue

Similarities in symptoms of fescue toxicosis and Cu deficiencies in ruminants have led to the examination of the relationship between the occurrence of *N. coenophalium* and Cu status in tall fescue. Saker et al. (1998) investigated the immune response and Cu status of yearling beef steers grazing E+ and low infected tall fescues. As a consequence

of grazing E+ tall fescue, immune function, including phagocytic activity, major histocompatibility complex class II expression and phagocytic activity, was decreased ($P < 0.05$) as well as ceruloplasmin. Also serum Cu was lower ($P < 0.05$) in animals grazing E+ (0.62 ppm) compared to those grazing E- (0.72 ppm). Similarly, Oliver et al. (2000) reported that over three grazing seasons serum Cu levels of steers grazing E+ were lower ($P = 0.003$) compared to E- (0.62 vs 0.72 μg/g, respectively). However, these animals had access to mineral containing Cu during the extent of this experiment, and therefore all effects on serum Cu cannot be attributed to the presence of the endophyte. Dennis et al. (1998) evaluated E+ and E- tall fescue varieties in greenhouse, plot, and pasture studies. Genetic clones or genetically similar fescue lines with the endophyte and without the endophyte were used in order to decrease the bias of plant Cu uptake due to genetic variation. In the greenhouse study, Cu levels were greater for E- compared to E+ in both genetic clones with the endophyte altered post emergence (3.4 vs. 2.8 μg/g, respectively; $P < 0.001$) and genetically similar strains with endophytes altered prior to planting (8.6 vs. 7.6 μg/g, respectively; $P < 0.05$). In field plots, endophyte only affected Cu concentration in September, E- being higher ($P < 0.05$) than E+ (7.3 vs. 6.6 μg/g, respectively). In pasture studies in Virginia, Cu level was affected by maturity and location. Copper levels were higher in E- compared to E+ in early summer regrowth and stockpiled forage. Dennis et al. (1998) suggested that Cu deficiencies in cattle grazing E+ fescue could be in part due to decrease intake of forages in addition to lower forage Cu content.

Due to the changes in Cu levels as experiments moved from greenhouse to pasture studies, it is apparent that Cu uptake by tall fescue plants may be controlled by a range of

factors such as plant genotype, endophyte strain, and environment (Dennis et al., 1998). Under P-limiting growing conditions Cu concentrations in roots were greater for E- compared with non-ergot alkaloid producing endophyte fescue (Malinowski et al., 2004). These authors previously demonstrated that phenolic-like exudates from the roots of E+ plants present in P-deficient medium and these compounds easily chelate Cu^{2+} and may immobilize it in soils with high organic matter content (Malinowski et al., 1998). Currently, there is no literature available on the effect of endophyte on Cu intake and bioavailability to the animal. Also, studies measuring Cu status in grazing animals typically report serum Cu. Liver is considered a better indicator of Cu status and currently there is no literature available reporting liver Cu in animals grazing tall fescue types.

Copper in metalloproteins

Biochemical research has shown Cu to be an essential component of several Cu-containing proteins that display oxidative reductase activity (Table 2-1). The function of Cu in these proteins has shown to be both as an electron transfer intermediate in redox reactions and an essential cofactor for catalytic activity (Uauy et al., 1998).

Superoxide dismutase exists in two forms: Cu/Zn and Mn. Copper/Zn superoxide dismutase (SOD) is localized in the cytoplasm where it catalyzes the dismutation of superoxide anions. During this catalysis, Cu in the active site of SOD is reduced with the substrate O_2^- and then H_2O_2 (Coblentz et al., 2000). In this reaction SOD acts as a free radical scavenger, thus helping organisms cope with oxidative stress. If the presence of the fungal endophyte in fescue limits the Cu available to the grazing animal, then there is potential for the level of SOD activity to be affected. If this is the case, there is potential for oxidative stress due to a decrease in SOD activity to play a role in fescue toxicosis.

Ward et al. (1993) reported no difference in erythrocyte SOD activity after 98 d in steers fed a Cu sufficient (11.2 ppm Cu) or deficient (6.2 ppm Cu) diet in combination with or without an additional supplement of Mo and S (10 ppm and 2%). Erythrocyte SOD is one of the last Cu-based enzymes affected by Cu deficiency due to its physiological importance (Paynter, 1987). Paynter (1987) hypothesized if Cu deficient diets continued for more than 98 d, SOD activity probably would be reduced. Ward and Spears (1997) fed bull calves either a Cu sufficient (11 ppm Cu) or deficient (7 ppm Cu) diet with (7 ppm Mo) or without (2 ppm Mo) molybdenum supplementation for 196 d. After 196 d there was a Cu x Mo interaction ($P < 0.01$) with highest SOD activity in bulls fed Cu without Mo (7.29 units/mg hemoglobin), lowest in animals receiving Mo without Cu (4.43 units/mg hemoglobin) and similar between bulls receiving no Cu and no Mo (6.92 units/mg hemoglobin) and bulls receiving Cu and Mo (6.86 units/mg hemoglobin).

Copper also plays a role as an important effector molecule regulating gene expression in eukaryotic organisms by activation and repression of gene transcription. Copper binds to regulatory metal-binding proteins in the nucleus of a cell. This binding affects the tertiary structure of these proteins, which become activated to interact with specific upstream regulatory elements in metal-responsive regulated elements (MRE) in the 5'- end of MRE-regulated genes (Furst et al., 1988). Amt1 and Ace1 are two Cu-dependent transcription factors that affect expression of the Sod1 gene (Uauy et al., 1998). Little information regarding metal-regulated transcription units and their copper-dependent activity in higher eukaryotes is available. Research comparing gene expression of these Cu-dependent enzymes in cattle grazing E+, E-, and non-ergot

alkaloid-producing endophyte-infected fescues could lead to understanding of the biointeractions which take place in cattle and cause fescue toxicosis.

INTAKE DETERMINATION

In grazing situations, the performance of ruminants is dependent on DMI, dry matter digestibility (DMD), and genetic capability. The relationship of intake to performance is more important due to the fact that grazing animals tend to select for or maintain diet quality at the expense of quantity (Lippke, 2002). As much as 60 to 90% of the variation in digestible energy intake may be contributed to animal variability, while 10 to 40% is due to diet digestibility (Crampton et al., 1960). In a survey of 114 trials with lactating dairy cows, diets with digestibility between 50 and 67 % were thought to limit intake through physical factors such as passage rate and digestibility. When digestibility increased above 67%, intake was limited by physiological factors (Conrad et al., 1964). The stage of maturity of forage can play an important role in intake regulation where intake is limited by physical (gut fill) mechanisms due to low digestibility and high fiber concentration. Laredo and Minson (1973) reported that bulk density of stem and voluntary intake are negatively correlated ($r = -0.70$) suggesting stage of maturity decreases voluntary intake.

Mechanisms controlling forage intake of ruminants are not simple. Forage intake is not simply controlled by one factor such as physical characteristics, rather mechanisms function through multiple interactions (Fisher, 2001). In grazing conditions, actual ingestive behavior (bite mass, bite rate, and grazing time) has been suggested to be more important in forage intake than physical or metabolic controls (Hodgson, 1982). This concept is more important in situations where forage availability and accessibility are low

due to interactions of grazing time, ingestive behavior, and canopy characteristics (Burns and Sollenberger, 2002). Forage intake is plant species-specific and is altered by plant maturity and morphology. Ruminants graze selectively and show a strong preference for lush green leaf against senesced and/or stem material (Minson, 1990). Given the opportunity to select for leafy material without limiting forage availability, the diet selected will increase above that on offer and intake will increase (Burns et al., 1991).

Measuring intake of forages

Due to the importance of measuring animal performance, the ability to measure or predict DMI and nutritive value has been the driving force behind studying the plant-animal interface (Burns and Sollenberger, 2002). A number of techniques have been developed to estimate intake due to the impracticality of measuring intake directly on pastures (Lippke, 2002). Moore and Sollenberger (1997) classified these techniques as follows: 1) estimate individual animal intake by fecal output and forage digestibility or ingestive behavior, and 2) estimate intake for groups of animals by disappearance of herbage mass, prediction from forage characteristics, or calculate from animal performance. Each method employs certain assumptions which must be met in order to validate the estimates.

"Cut and carry" techniques where fresh forage is harvested and offered to animals have been used with animals in total confinement to reduce errors associated with estimations of forage intake on pastures. Though this method allows for exact assessment of forage consumed, it reduces the animal selection and does not representing grazing conditions.

Fecal output and forage digestibility have been used extensively to calculate DMI using the following equation:

$$DMI = \text{fecal output}/(100\text{-}DMD)$$

Fecal bags have been used for total fecal collection in grazing situations to measure total fecal output. This method poses several problems including potential loss of material and/or urine contamination, but more importantly, altering grazing behavior (Buntinx et al., 1992).

A technique that helps alleviate the errors associated with total collection is measuring fecal output through marker technology. Markers can be classified as internal or external and to properly measure fecal output should be: 1) unabsorbable, 2) unaffected by and have no effect upon animal or microbial digestive processes, 3) evenly distributed throughout and having the same passage rate as the digesta, and 4) analyzable with specific and sensitive methodology (Owens and Hanson, 1992). Although some markers have several of these characteristics, no marker fully meets all of these criteria.

Chromium sesquioxide (Cr_2O_3), also known as chromic oxide, is the most widely used external marker for intake estimation over the past 50 yr (Lippke, 2002). The major problem encountered with its use involves the movement of Cr_2O_3 through the digestive tract being independent of undigested particles from the diet. This can cause a strong diurnal variation in the fecal Cr_2O_3 concentration. Therefore, to accurately estimate intake with Cr_2O_3, a constant intake of Cr_2O_3 along with frequent fecal sampling is required. This increase in animal handling could alter normal animal grazing behavior. Alternatively, France et al. (1988) reported that a method using a single dose of Cr_2O_3 followed by numerous fecal collections until the labeled source clears reduces the need for numerous dosing. From these data, a nonlinear equation relating time after dose to fecal Cr_2O_3 is formulated to estimate fecal output.

Buntinx et al. (1992) evaluated the use of controlled-release devices (CRD) and suggested that they could reduce labor and possibly prevent negative alterations to grazing behavior. With-in day coefficient of variation of fecal concentration has shown to be reduced in sheep and cattle by the use of CRD (Adams et al., 1991). However, with the use of CRD, total fecal collection is required on a subset of animals to validate the release rate defined by the manufacturer.

Alkanes as markers

Oró and Nooner (1965) reported on similarities between the alkane patterns of cattle feces and that of herbage consumed. However, this study did not intended to investigate relationships to DMI, and its importance went unnoticed for several years. It was not until 16 yr later that Grace and Body (1981) first suggested that the cuticular wax component of plants could be used as markers for nutritional studies. These researchers demonstrated that long-chained fatty acids (C_{19}-C_{32}) were quantitatively recoverable in the feces. The later work of Mayes and colleagues was the first to investigate alkanes as possible markers (Mayes and Lamb, 1984; Mayes et al., 1986b, , 1988; Dove, 1991). Although minor components, alkanes are completely saturated aliphatic hydrocarbons ubiquitous to the cuticular wax of higher plants. The linear n-alkanes are the predominant form found in plants ranging in length from 21 to 37 carbon atoms. The most common alkanes found in plants are the odd-number chained alkane, predominantly nonacosane (C_{29}), hentriacontane (C_{31}), and tritiaconane (C_{33}) (Dove, 1991).

Mayes et al. (1986b) reported incomplete fecal recovery of alkanes; however, these authors suggested that this obstacle could be over come by dosing animals with synthetic, even-chained alkanes as external markers for fecal output. Given the pair of natural (odd-chained) and synthetic (even-chained) had similar recovery, the errors associated

with the incomplete recoveries would cancel out in numerator and denominator (Mayes et al., 1986b). In subsequent research, alkanes adjacent in chain length have shown similar recovery rates in sheep (Vulich et al., 1991; Dove and Olivan, 1998; Dove et al., 2002) and in cattle (Unal and Garnsworthy, 1999; Berry et al., 2000). Therefore, daily intake by grazing animals can be calculated with the following equation:

$$DMI = \left(\frac{Fi}{Fj} * Dj\right) \Big/ \left(Hi - \frac{Fi}{Fj} * Hj\right)$$

Where:

DMI = Daily herbage intake (kg DM/d)

Dj = daily dose of even-chained alkane

Fi and Hi = fecal and herbage concentration of odd-chained alkane

Fj and Hj = fecal and herbage concentration of even-chained alkane

Dosing and sample collecting

In studies conducted on sheep using gelatin capsules and paper pellets as matrix for the dosed alkanes, the dosed alkane reached equilibrium in fecal concentration in 5-6 d (Mayes et al., 1986b; Dove, 1991). As previously mentioned with chromic oxide as a marker, diurnal variation in fecal recovery could result in biased intake estimations. In studies dosing twice daily with alkanes, discrepancies between actual and estimated herbage intake were higher in PM-collected fecal samples compared to AM-collected samples (Hameleers and Mayes, 1998a; Berry et al., 2000). Mayes et al. (1988) suggested that these discrepancies could be due to the tendency of plant alkanes to associate with the particulate phase and dosed alkanes to associate with the liquid phase of digesta.

Fecal recovery of alkanes increases as carbon-chain length increases, and as chain length increases, differences in recovery rates of adjacent alkanes decreases (Table 2-2; Table 2-3). The fecal recovery rates of C_{32} and C_{33} have historically been very similar, and thus the ratio of these two alkanes has been used to correct for incomplete fecal recovery (Vulich et al., 1991; Unal and Garnsworthy, 1999; Berry et al., 2000; Dove et al., 2002).

Lopez-Guerrero (2005) reported the daily DMI of steers fed fescue hay using chromic oxide with AM or PM fecal sampling (4.72 and 4.72 kg, respectively) was similar ($P = 0.99$) to the actual DMI (4.73 kg) Similarly, Hamaleers and Mayes (1998b) reported that the estimated daily DMI of dairy cattle consuming ryegrass silage using the alkane ratio of two naturally occurring alkanes (C_{27}:C_{35}) was similar($P = 0.34$) in AM (6.7 kg) and PM (7.0 kg) compared to group actual intake (6.8 kg/head). When using the ratio of C_{27} to the dosed alkane C_{36}, these authors reported that DMI estimation was higher ($P < 0.001$) in AM sampling (9.6 kg) compared to PM sampling (7.8 kg). These differences are explained by diurnal variation in C_{36} excretion being greater than the natural alkane, C_{27} (Hameleers and Mayes, 1998b). Berry et al. (2000) reported that DMI of forage (unspecified species) using C_{32} and C_{33} alkanes did not differ due to sampling time. Estimates of DMI of Brown Swiss cows fed in confinement did not differ when samples were collected at 0630, 1330, and 2030 (15.4 ± 0.19, 18.5 ± 2.0, and 17.3 kg ± 1.2 kgDM/d). Lack of differences between sampling times was likely due to large variation. Berry et al. (2000) suggest that 0630 was the optimal time for fecal sampling due to the smallest difference compared to actual DMI (15.3 kg ± 0.19 DM/d).

Botanical composition

Alkanes can be used to partition the total intake into plant species in mixed swards, or plant parts in homogeneous swards (Dove and Moore, 1995). Most plant parts and plant species have a characteristic pattern of alkanes. Dove et al. (1999) showed this by measuring the alkane pattern in plant parts, espophageal extrusa of fistulated sheep, and in feces of both fistulated and non-fistulated sheep. These authors were able to determine animals shifted their dietary preference from leaf to stem by spraying annual grasses with glyphosate. Dove and Moore (1995) suggested that the ability to characterize the botanical composition of the diet will improve as our understanding and ability to analyze multiple plant wax components progresses.

Intake estimation

Scaglia et al. (2005) estimated DMI of steers grazing E+ tall fescue and E+ tall fescue/alfalfa (*Medicago sativa* L.) using both total collection with fecal bags and rectal grab samples. Recovery of C_{32} from bag samples and grab samples was similar with an average ratio (grab:bag) of 0.92 and 0.99 for fescue and fescue/alfalfa. Average DMI of steers was similar ($P > 0.05$) on fescue and fescue/alfalfa (10.8 vs.10.3 kg DM). These authors reported that the fecal recovery rate of C_{32} in feces was 0.991 and 0.985 for steers grazing fescue and fescue/alfalfa, respectively; higher than previous reported recovery rates (Mayes et al., 1986b; Dove and Mayes, 1991). Lopez-Guerrero (2005) reported that estimated average daily DMI of steers grazing E+ tall fescue using C_{32} and C_{33} alkanes to be higher ($P < 0.01$) than actual intake (6.17 vs 4.74 kg DM). However these authors reported an unusually high average recovery rate of C_{33} compared to C_{32} (1.30 vs. 0.81) possibly causing the overestimation of intake. Berry et al. (2000) conducted a digestion trial to measure DMI of Brown Swiss dairy cows fed unspecified forage using an alkane

controlled release device containing C_{32} and C_{36}. These authors reported that the pair of alkanes C_{32}/C_{33} more closely estimated DMI (12.67 kg DM) compared to actual intake (12.70 kg DMI) due to similar fecal recovery rates (0.87 and 0.85, respectively).

Hameleers and Mayes (1998a) measured forage intake in dairy cows using the dosed C_{32} alkane and the fecal ratio of C_{32}:C_{33}. Cows were fed 8, 10, 12, or 14 kg DM/d perennial ryegrass and white clover with or without 2 kg DM/d of barley. The inclusion of barley did not effect ($P > 0.05$) estimated DMI compared to actual DMI. Across diets, differences between calculated and actual DMI ranged from 139 to 366 g DM/d. Unal and Garnsworthy (1999) examined intake of confined dairy cows consuming a hay [perennial ryegrass (*Lolium perenne* L.), timothy (*Phleum pretense*), meadow fescue (*Lolium pratense*), and orchardgrass (*Dactylis glomerata*] or perennial ryegrass silage with the use of the dosed alkanes C_{32} and C_{36} in combination with C_{33} as an internal marker. Across the range of actual intakes (6-24 kg DM/d), r^2 values of actual versus estimated DMI ranged from 0.81 to 0.99. Dry matter intake of hay diets was overestimated, on average, with C_{33}:C_{32} (8.24 kg DM/d) and underestimated with C_{33}:C_{36} (7.94 kg DM/d) compared to actual (8.13 kg DM/d). Both ratios overestimated DMI of silage diets, but produced a similar estimate of DMI (8.09 kg DM/d) compared to actual DMI (7.91 kg DM/d).

TABLES AND FIGURES

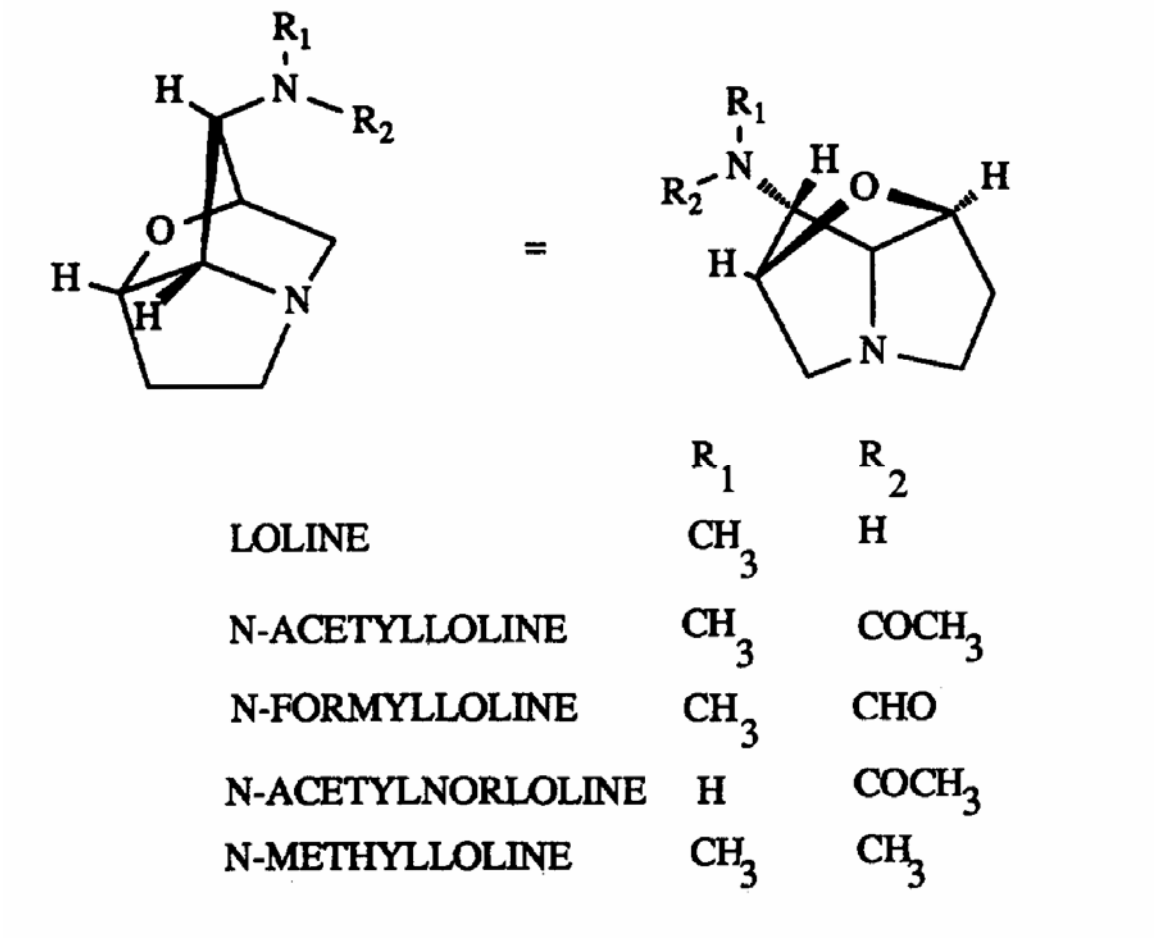
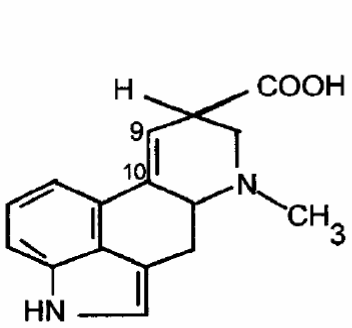
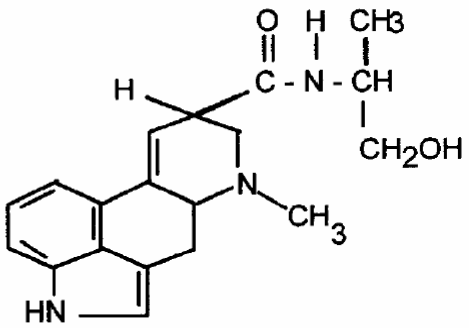


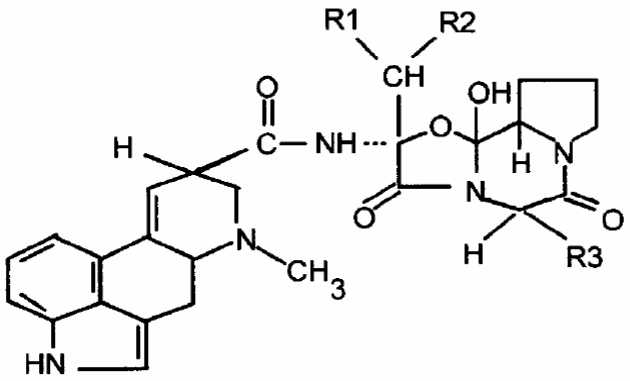
Figure 2-1. Loline alkaloids isolated from *N. coenophialum*-infected tall fescue (Adapted from Porter, 1995).



Lysergic acid



Ergonovine
(Lysergic acid amide)



Ergopeptine alkaloids

R1	R2	R3		
		$-CH_2C_3H_5$	$-CH_2CH(CH_3)_2$	$-CH(CH_3)_2$
H	H	Ergotamine	Ergosine	Ergovaline
CH_3	CH_3	Ergocristine	Ergocryptine	Ergocornine
H	CH_3	Ergostine	Ergoptine	Ergonine

Figure 2-2. Chemical structure of ergot alkaloid classes (Stuedemann et al., 1998).

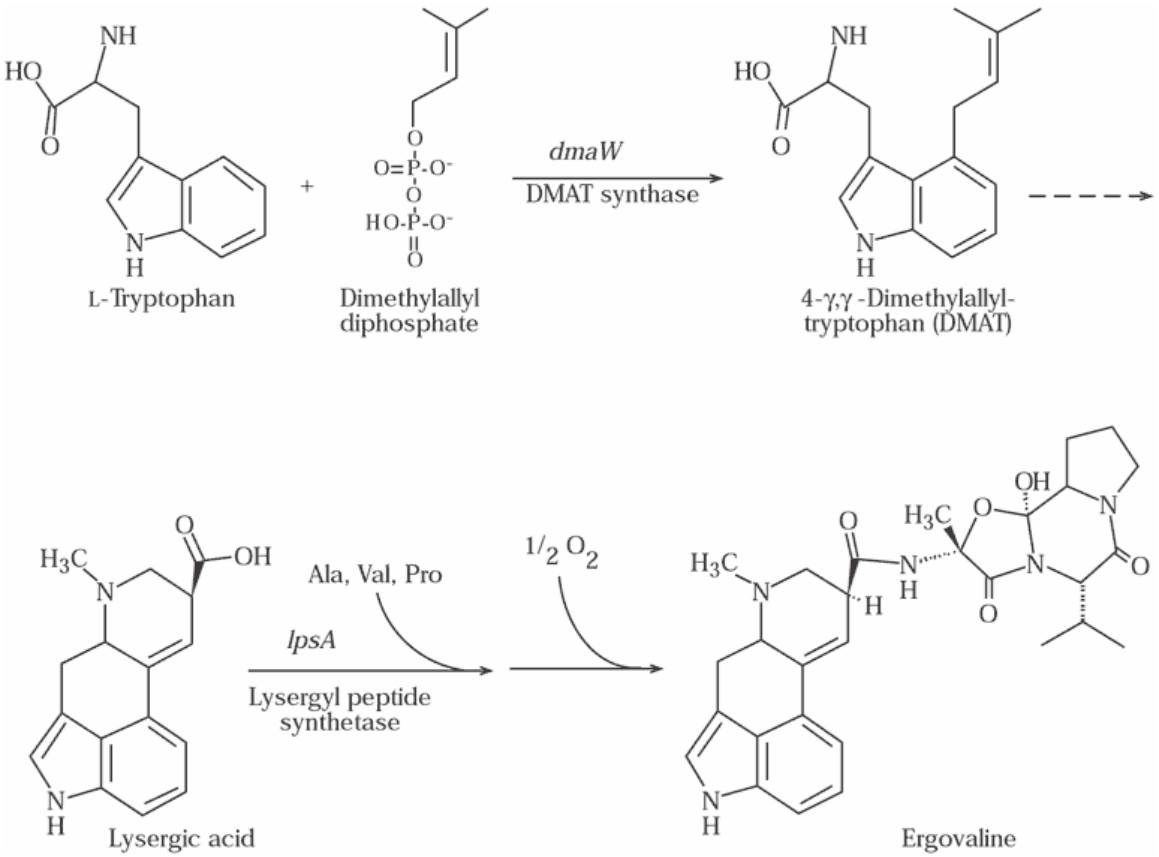


Figure 2-3. Steps in ergovaline synthesis (adapted from Schardl, et al., 2002).

Table 2-1. Functions of cuproenzymes with oxidation and reduction ability

Enzyme	Function
Superoxide dismutase	Superoxide dismutation
Cytochrome-*c* oxidase	Electron transport, terminal oxidase
Catechol oxidase	Melanin Synthesis
Ceruloplasmin	Ferroxidase
Amine oxidases	Deamination of primary amines
Dopamine-β-hydroxylase	Dopamine → norepinephrine
Protein-lysine 6-oxidase	Collagen and elastin cross-linking
Peptidylglycine hydroxylase	A-Amidation of neuropeptides

Table 2-2. Recovery rates (%) of alkanes from sheep

	Alkanes					
Forage species	C_{31}	C_{32}	C_{33}	C_{35}	C_{36}	Reference
Lolium perenne[a]	83	--	91	97	--	(Mayes and Lamb, 1984)
L. perenne	85	89	89	93	--	(Mayes et al., 1986a)
L. perenne	78	86	84	95	92	(Dove and Mayes, 1991)
L. perenne	93	88	88	91	86	(Vulich et al., 1991)
L. arundinacea	86	90	83	--	--	(Piasentier et al., 1995)
L. perenne[b]	93	96	95	--	99	(Dove and Olivan, 1998)
L. perenne	80	94	93	103	98	(Dove et al., 2002)

[a] Mixed with *Trifolium repens*
[b] Supplemented with sunflower mill

Table 2-3. Recovery rates (%) of alkanes from cattle

	Alkanes				
Forage species	C_{31}	C_{32}	C_{33}	C_{36}	Reference
Lolium perenne	59	77	81	93	(Mayes et al., 1986b)
Mix of hays	--	95	94	95	(Unal and Garnsworthy, 1999)
Mix of forages	76	87	85	81	(Berry et al., 2000)
Bromus riparius	59	84	63	83	(Moshtaghi and Wittenberg, 2002)

CHAPTER 3
ANIMAL RESPONSE AND ALKALOID DETERMINATION OF STEERS GRAZING THREE FESCUE TYPES AND LAKOTA PRAIRIE GRASS

ABSTRACT

Tall fescue [*Schedonorus arundinaceus* (Schreb.) Dumort.] is associated with deleterious effects on grazing animals due to ergot alkaloid production by the fungal endophyte *Neotyphodium coenophialum*. The objectives of this research were to evaluate animal response of steers grazing 'Kentucky-31' endophyte-infected (E+) and endophyte free (E-) tall fescues, Q4508-AR542 non-ergot alkaloid-producing endophyte infected tall fescue (Q), and 'Lakota' prairie grass (L; *Bromus catharticus* Vahl.) and the presence of alkaloids in forage and ruminal fluid of steers. All forages were evaluated in 2004 and only fescue types in 2005. In 2004, steers' ADG were higher ($P < 0.05$) on E-, Q and L compared to E+. Across years, ADG of steers grazing E- and Q were higher ($P < 0.05$) than for steers grazing E+ and there was a trend ($P = 0.10$) for ADG of steers grazing E- to be higher than for those grazing Q. In 2004, diurnal body temperatures of steers grazing Q, L, and E+ were monitored for 5 d approximately every 28 d with a tympanic temperature logger. During these periods there was no difference ($P > 0.10$) in steers' temperature. Lysergic acid amide (LSA), an analog of lysergic acid, and ergovaline were present in E+ forage throughout the grazing season but not detectable in E-, Q, and L. Similarly, LSA appeared in ruminal fluid of steers grazing E+, but not in steers grazing E-, Q, and L. Ergovaline was not detectable in ruminal fluid of steers grazing any of the four treatments. The appearance of LSA in ruminal fluid through the season was similar

to patterns of forage alkaloids (LSA and ergovaline). These results suggest that E-, Q, and L are potentially beneficial forage crops to replace or compliment E+, however due to lower performance of animals grazing Q, the tall fescue cultivar Q4508 may not be the best fescue type for the incorporation of non-ergot alkaloid-producing endophyte strains. Additionally, the appearance of LSA in ruminal fluid of steers grazing E+ suggests that this ergot alkaloid may contribute to fescue toxicosis.

INTRODUCTION

Tall fescue [*Schedonorus arundinaceus* (Schreb.) Dumort.] is extensively used as a forage crop throughout the southeastern United States covering approximately 14 million ha of land (Bacon and Siegel, 1988). The fungal endophyte (*Neotyphodium coenophialum*), which naturally infects tall fescue, has been associated with the deleterious effects on grazing animals generally referred to as fescue toxicosis (Stuedemann and Hoveland, 1988). This endophyte produces alkaloids that are considered to cause this disorder, but it is also responsible for helping tall fescue cope with biotic and abiotic stresses (Hoveland, 1993). Attempts have been made to remove the endophyte (Hoveland et al., 1982) or use a non-ergot alkaloid-producing endophyte infected tall fescue (Bouton et al., 2002) but, this can result in decreased stand persistence or be too costly for producers. Ergovaline, the most abundant ergot alkaloid in tall fescue has been previously identified as the toxic compound causing fescue toxicosis (Porter, 1995). However, studies investigating absorption potential across the reticulum, rumen, and omasum and urinary appearance and clearance have suggested that simpler ergot alkaloids are the causative compounds (Stuedemann et al., 1998; Hill et al., 2001). If the exact alkaloids available for absorption from the rumen can be identified, pharmacological methods may be developed to prevent absorption and allow producers to

continue benefiting from the positive agronomic properties of alkaloid-producing endophyte-infected tall fescue.

Lysergic acid amide (LSA) is a simpler ergot alkaloid in E+ tall fescue (Petroski and Powell, 1991) that has shown to have vasoconstrictor activity in bovine vasculature (Oliver et al., 1993). Lodge-Ivey et al. (2006) reported that lysergic acid, an analog of lysergic acid amide, was detectible in ruminal fluid However, currently no literature is available on the presence of LSA in the ruminal fluid of animals grazing alkaloid-producing endophyte-infected (E+) tall fescue. The objectives of this research are to evaluate the animal responses to grazing three fescue types and 'Lakota' prairie grass (L; *Bromus catharticus* Vahl.) and the presence of alkaloids in forage and ruminal fluid of steers.

METHODS AND MATERIALS

Treatments and design

Treatments were defined as forage type and included alkaloid-producing endophyte-infected Kentucky 31 tall fescue (E+), endophyte-free Kentucky 31 tall fescue (E-), non-ergot alkaloid-producing endophyte (strain AR542) infected Q4508 tall fescue (Q), and Lakota prairie grass (L). In 2005, emphasis was placed on differences in fescue types and L was not evaluated. Treatments were arranged in three replicates of a randomized complete block design with pasture as the experimental unit.

Pasture and animal management

Pastures were managed under rotational stocking during the grazing seasons of 2004 (135 d) and 2005 (136 d). Grazing began on May 5 and May 3 for 2004 and 2005, respectively. Each pasture was subdivided into six paddocks (approximately 0.20 ha), and animal movement from paddock to paddock was determined by available forage

(based on residual height of approximately 7 to 10 cm). Pastures were seeded September 20 to 25, 2002 at seeding rates of 39 kg/ha for Lakota and 25 kg/ha for all the fescues. Due to stand failure, the E- treatment was reseeded on March 30, 2003. All pastures were fertilized according to soil test recommendations. Prior to the beginning of the grazing season of both years 33.6 kg/ha of liquid N was applied to all treatments. In 2004, an additional 56 kg/ha of 46-0-0 fertilizer was applied to all treatments on August 20 and to L after each of the sub-paddocks were grazed by the steers for 7 to10 d. Hay was harvested from three of the six paddocks in each pasture on June 6 (2004) and May 15 (2005). The remaining paddocks were clipped after animals were removed in late spring to remove reproductive tillers.

For the 2004 grazing season, steers were purchased on December 1, 2003 at a Virginia feeder cattle sale and shipped to Smithfield Farm, Virginia Tech, Blacksburg, VA. Steers were kept in drylot from the date of purchase until May 4, 2004. From January 6 to May 4, steers were fed a diet consisting of 51% barley straw, 37% corn, and 5% molasses, with the remaining portion containing soybean meal, feather meal, and urea. Average daily gain (ADG) for steers during the drylot period was 0.64 kg. In 2005, steers were purchased on April 19, 2005 at a Virginia feeder cattle sale and shipped to Kentland Farm, Virginia Tech, Blacksburg, VA. Thirty-six crossbred steers with initial average BW of 272 ± 19 kg in 2004 and 244 ±17 kg in 2005 were blocked by weight and randomly allotted within block to the four treatments. Three steers were in each of the pastures, two of which were randomly assigned as tester animals for sampling. In both years, steers did not have access to mineral supplementation to limit Cu intake to only that from the forage. After purchasing of steers each year, steers were vaccinated for

Infectious Bovine Rhinotracheitis (IBR), Bovine Viral Diarrhea (BVD), Bovine Respiratory Syncitial Virus (BRSV), Parainfluenza3 (PI3) with Pyramid 4® (Fort Dodge Animal Health, Fort Dodge, IA) and with Vision 7® (Intervet, Millsboro, DE) for Clostridium chauvoei (Blackleg), C. septicum (Malignant edema), C. novyi (Black disease), C. sordellie and C. perfringens Types C and D (Enterotoxemia). Steers were treated with Cydectin® (moxidectin; Fort Dodge Animal Health, Fort Dodge, IA) for internal and external parasites on d 0 and 56 of the grazing season and with Elector® (spinosad; Elanco™ Animal Health, Greenfield, IN) as needed for external parasites. On approximately d 56 of both years animal were treated with Co-Ral Plus® insecticide ear tags (Diazinon and Coumaphos; Bayer HealthCare, Shawnee Mission, KS).

Animals and forage samples

On d 0 and every 28 d thereafter, steers were gathered at between 0700 and 0900 and weighed (unshrunk), rectal temperatures recorded, and ruminal fluid collected. Ruminal fluid was placed on dry ice and transported to the laboratory where stored at -20 °C until further analysis.

During the 2004 grazing season, diurnal body temperature (2 steers per treatment) was monitored approximately every 28 d with a tympanic temperature logger (Onset Computer Corp., Pocasset, MA). Due to concurrent research with other monitoring devices, only E+, Q, and L treatments were monitored for diurnal temperature. Data loggers recorded body temperature at 15-min intervals for a 5-d period. Data were analyzed using Boxcar Pro® 4.3 (Onset Computer Corp., Pocasset, MA). No shade was available for cattle throughout the experiment. Ambient temperature, relative humidity, and precipitation were recorded daily at the research station (VAES, 2006). Heat stress

conditions were measured using the Temperature Humidity Index (THI) which was calculated using the following equation (NOAA, 1976):

$$\text{Air Temperature } ^{o}\text{F} - (0.55 - (0.55 * \text{RH\%} / 100)) * (\text{Air Temp } ^{o}\text{F} - 58.8)$$

On days the steers were weighed, hand-plucked forage samples (collected from approximately the top 8 cm of the canopy by hand to mimic the selectivity of the forage exerted by the grazing animal) were collected from the sub-paddocks the steers would enter for nutritive value analysis. Plant material was dried in a forced-air oven at 60 °C for 48 h and ground through a 1-mm screen in a stainless steel Wiley Mill (Thomas Wiley Laboratory Mill Model 4, Arthur H. Thomas Co., Philadelphia, PA). Herbage mass (HM) was measured every 28 d prior to animals entering a new paddock. Herbage mass was measured by clipping three 0.25-m^2 quadrants to a stubble height of 2 cm and drying material at 60 °C for approximately 72 h (Rotz, 2006).

Hand-plucked samples used for DMI estimation in Chapter 4 were analyzed for ergot alkaloid concentrations. Briefly, during three periods throughout the grazing season, hand-plucked samples were collected over four alternating days for alkaloid analysis, placed on ice for transport to the laboratory, and frozen at -20 °C until further analysis. Rate of endophyte infection was assayed on tall fescue tillers collected at the end of the 2004 grazing season using an immunoblot procedure (Hiatt et al., 1997).

Laboratory analysis

Forage samples collected for nutritive value were dried in a forced-air oven at 60 °C for 48 h and ground to pass a 1-mm screen in a stainless steel Wiley Mill (Thomas Wiley Laboratory Mill Model 4, Arthur H. Thomas Co., Philadelphia, PA). Ash was determined by placing samples in a 500 °C muffle furnace for 3 h (AOAC, 2000). Crude

protein was determined by analyzing N content with a Nitrogen Auto-analyzer (Perkin Elmer 2410 N Analyzer, Norwalk, CT) by the combustion method (AOAC, 2000). Neutral detergent fiber and ADF were analyzed with an Ankom 200/220 Fiber Analyzer (Goering and Van Soest, 1970).

Samples collected for alkaloid analysis were lyophilized in a FreeZone 12 L lyophilizer (Labconco, Co., Kansas City, MO) or a VirTis Genesis 25 EL lyophilizer (SP Industries, Inc., Gradiner, NY). Dried samples were ground to pass a 1-mm screen in a Wiley mill (Thomas Wiley, Laboratory Mill Model 4, Arthur H. Thomas Co. Philadelphia, PA) and then a 0.5 mm screen in a Tecator Cyclotec 1093 sample mill (Tecator, Hogänäs, Sweden). Samples were analyzed for ergovaline and lysergic acid amide as described by Hill et al. (1993) with slight modification (Rottinghaus, G.E., University of Missouri, Columbia, MO, personal communication). Briefly, 5 g of ground forage was weighed into polypropylene screw cap bottles along with 100 mL chloroform and 5 mL 0.1 M sodium hydroxide. Samples were mixed overnight (approximately 18 h) on a rotator shaker. After mixing, 2 g sodium sulfate was added and samples were mixed for an additional 30 min. Twenty milliliters of extract were then filtered through Whatman PS-1 filter paper (Whatman Inc., Florham Park, NJ) followed by 10 mL of extract applied to an ergosil cleanup column under vacuum. Cleanup columns were prepared by placing a 12.7-mm biological disk (Whatman Inc., Florham Park, NJ) in the bottom of a 6-mL disposable syringe barrel followed by: a) 1 mL ergosil (Analtech, Inc., Newark, DE); b) a 12.7-mm biological disk; c) 1 mL ground sodium sulfate; and d) a 12.7-mm biological disk. Pigments were removed by washing the columns with 1.8 mL acetone:chloroform (8:2) followed by 3 mL petroleum ether under vacuum. Ergot

alkaloids were then eluted with methanol to give a final volume of 2 mL. The methanol eluent was passed through a small Romer Mycosep™ 224 column (Romer Labs Inc., Union, MO) to remove the remaining pigmentation prior to HPLC analysis. Ergovaline and lysergic acid amide were determined by HPLC with fluorescence detection. Standards for each alkaloid (50, 100 ppb) were prepared in methanol. Methanolic samples were loaded onto an autosampler (Perkin Elmer ISS200, Norwalk, CT) and 20 µL was injected into a Perkin Elmer (LC-250) HPLC pump with a 100 x 4.6 mm (3- µm particle size) Luna C_{18} analytical column and detected with a fluorescence detector (excitation 250 nm, emission 420nm; Hitachi F-1200; Hitachi High Technologies America, Inc., San Jose, CA). The mobile phase (30% acetonitrile in a 200 mg/L solution of ammonium carbonate in distilled water) was pumped at a rate of 1 mL/min.

Statistical analysis

Seasonal data were analyzed as a randomized complete block design with forage as treatment. Due to the evaluation of the L treatment only in 2004, these data were only used in analysis of the 2004 data and not in across year analyses. Data were analyzed using repeated measures analysis of variance in PROC MIXED with treatment as a fixed effect and year as the repeated variable for across year analysis and sampling date as the repeated variable for seasonal trends. All means were reported as least squares means. The compound symmetry, cs, covariance structure provided the best fit data for analyses as compared to unstructured, un, and autoregressive, ar(1). Differences were determined for the repeated measures using LSD adjusted *P*-values.

RESULTS AND DISCUSSION

Forage characteristics

Mean rate of endophyte infection exceeded 82% for E+ and 90% for Q and was not detectable in E-. Seasonal rainfall data are presented in Table 3-1. Precipitation was above average in 2004, and drier than average in 2005. Forage (E-, Q and E+) nutritive values (NDF, ADF, and CP) were not different between years (Table 3-2). In 2004, ADF was significantly higher in L compared to E+. However this difference (1.3%) is not biologically significant.

Allen et al. (2001) reported similar values of NDF (57.4 to 61.6 %) but CP (19.5 to 21.8%) was higher in summer-grazed E+ tall fescue than those reported in the current research. McCracken et al. (1993) reported higher values of NDF (67.0 to 70.5 %) and ADF (55.7 to 56.5 %) on E- tall fescue. These pastures were continuously stocked at a stocking rate of 94 kg/ha. The combination of continuous stocking and low stocking rate likely increased the accumulation of mature plant material, increasing the fiber fraction. Dubbs et al. (2003) reported higher levels of NDF (61 to 78 %), slightly higher values of ADF (30 to 41 %) and similar CP (9 to 16 %). The nutritive value of tall fescues in the present study were similar to those reported by the NRC. Values are not available for L in the NRC (2000). However, Lowe et al. (1999) reported 'Matua' prairie grass (*Bromus willdenowii* Kunth. cv. 'Grasslands Matua'), a similar species to L, to have NDF values (58.7 to 65.5 %) slightly higher on average than L in this research.

There was a year effect ($P<0.05$) on HM (3030 and 3520 kg/ha for 2004 and 2005, respectively). Interestingly, HM was higher in 2005 when rainfall was 235 mm below the 11-yr average compared to 2004 when the total rainfall was 25 mm above the 11-yr average. The difference in size of steers between years may contribute to the difference

in HM. Initial BW of steers in 2005 was 40 kg lighter than 2004. This resulted in an initial stocking rate of 820 kg BW/ha in 2004 compared to 725 kg BW/ha in 2005. During the 2004 season HM of E+ and Q were higher than L while all the fescue types were similar between them (Table 3-2). In 2005, there were no differences in HM among treatments, while across seasons both E+ and Q were higher than E- (Table 3-2). Herbage mass previously reported has varied likely due to differences in forage management. Parish et al. (2003b) reported that herbage mass of three fescue types (Kentucky 31 E+, E-, and AR542-infected fescue) grazed by stocker cattle in north Georgia ranged from 1710 to 2080 kg/ha in the fall and 1760 to 2020 kg/hain spring. In central Georgia, HM ranged from 2820 to 3060 kg/ha in the fall and 2780 to 3690 in spring (Parish et al., 2003b). Bouton et al. (2002) reported that herbage mass of Jesup E+, E- and AR542 grazed by sheep in spring were similar and ranged from 2150 to 2540 kg/ha.

Animal response

Average daily gains

In 2004, ADG were higher in steers grazing L compared to all fescue types (Table 3-3). Across years, ADG of steers grazing E- and Q were higher compared to those grazing E+. These differences are consistent with those observed by Parish et al. (2003b) and Nihsen et al. (2004). Parish et al. (2003b) compared ADG of steers grazing three types of Kentucky-31/ Jesup-5 tall fescue treatments (AR542 non-ergot alkaloid-producing endophyte infected, E-, and E+) at two locations in Georgia (central and northern) over 3 yr. In north Georgia, ADG of steers grazing E- and AR452 were higher ($P < 0.05$) in both autumn and spring for E- and AR542 (0.84, 0.82, 0.71, and 0.73 kg, respectively) compared to E+ (0.41 and 0.49 kg for autumn and spring, respectively). In

central Georgia ADG of steers grazing E- and AR542 (0.87 and 0.81 kg, respectively) were higher ($P < 0.05$) than E+ (0.56 kg) in autumn. In spring, gains were greatest (0.97 kg/d; $P < 0.05$) with E-, intermediate wtih AR542 (0.78 kg/d) and least with E+ (0.31 kg/d) in central Georgia (Parish et al., 2003b). Nihsen et al. (2004) reported ADG of steers grazing Kentucky 31 E+ tall fescue, HiMag E-, and HiMag E- infected with two strains of ergopeptine-deficient endophytes (HiMag 4 and HiMag 9) in northern Arkansas and southern Missouri. Across locations, ADG was higher ($P < 0.05$) for steers grazing E-, HiMag4 and HiMag9 (0.61, 0.60 and 0.54 kg, respectively) compared to those grazing E+ (0.34 kg).

Average daily gains in steers grazing E- were greater than those grazing Q. Across both seasons, DMI of steers grazing E- (22.2 g/kg BW) was higher ($P < 0.01$) than for those grazing Q and E+ (18.6 and 19.4 g/kg BW, respectively). Decreased gains of animals grazing E+ compared to E- has been attributed to decreased DMI caused by fescue toxicosis (Paterson et al., 1995; Abaye et al., 2002; Parish et al., 2003a; Parish et al., 2003b). The decreased gains on E+ in the present study are likely due to reduced DMI. Interestingly, ADG of steers grazing Q tended ($P = 0.11$) for to be lower than those for steers grazing E-. This may partly be explained by decreased DMI. However, ADG was higher in steers grazing Q compared to those grazing E+ while DMI was similar. Peters et al. (2002) reported that cows with nursing calves grazing E+ lost more ($P < 0.05$) BW during August compared to cows on a low-endophyte tall fescue (46 vs. 16 kg) while DMI was similar (16 vs. 19 g/kg BW). These authors suggested that lower performance of cattle grazing E+ tall fescue may not be solely the result of decreased DMI, but also due to problems associated with altered efficiency of nutrient utilization.

The indigestible fraction of Q (4.0 %/h) was higher ($P < 0.05$) compared to both E+ and E- (2.2 and 2.5 %/h, respectively; Scaglia, personal communication). This would suggest a lower passage rate and possibly explain the decreased DMI of Q compared to E-, but does not support the suggestion of Peters et al. (2002) regarding altered nutrient utilization in E+. Hemken et al. (1981) suggested environmental temperature and fescue toxicosis interact where higher temperatures increase the ill effects of fescue toxicosis compared to lower temperatures. Temperature Humidity Index (THI) indicates animals were exposed to mild and medium stress during each grazing season of the present study. Therefore, although DMI was similar between E+ and Q the interaction of environment conditions and fescue toxicosis likely resulted in lower ADG.

Average daily gains of steers in May 2005 were higher compared to the remaining of the season and the 2004 season (Table 3-4). In 2005, steers started the grazing experiment directly after being purchased from a feeder cattle sale. These high ADG compared to the remaining grazing season and those in 2004 are likely caused by compensatory gain. Steers entering yearling programs are nutritionally restricted for various times and to varying degrees and therefore show compensatory gains during stockering/backgrounding programs (Klopfenstein et al., 1999). Also during May of both years forage was low in fiber concentration (NDF and ADF) and high in CP (Tables 3-5 and 3-6). In June, ADG decreased in both years but in 2004 steers did not gain weight. Low gains during this time may be attributed to forage being in a reproductive stage in late spring and structural carbohydrates increasing and CP decreasing. These changes are associated with the presence of reproductive tillers (Nelson and Moser, 1995). Also hay production on half of the paddocks resulted in insufficient available herbage during both

years. Therefore, steers did not graze these paddocks in sequential order, but were rotated to pastures previously grazed. These paddocks were mechanically clipped to remove stems and inflorescence after their initial grazing. The residual stubble height (approximately 20 cm) allowed for more residual mature and senescent material to remain.

In July 2004, ADG increased in all treatments. During this time, steers were moved to paddocks where hay had been harvested earlier in the season. Hay production removes herbage to a lower stubble height (approximately 7 cm), thus removing accumulated mature material. Although herbage mass was lower, the regrowth had less fiber and more CP compared to that of the previously grazed paddocks (Table 3-5). In July 2005, ADG was close to zero. Hay production in 2005 occurred 22 d later than in 2004 and rainfall was below average (Table 3-1). Inadequate rainfall and rest period resulted in insufficient HM on these paddocks. Therefore, animals were still grazing paddocks similar to those described in June causing the lower ADG.

In August 2004, ADG decreased in all treatments. Steers were rotated back to paddocks that were clipped to a higher stubble height earlier in the season. Although CP was similar, fiber concentrations increased compared to previous periods (Table 3-5). In August of 2005, steers were moved to paddocks where hay was previously harvested. Nutritive value was similar to previously grazed paddocks (Table 3-6), but differences in canopy structure may have affected animal selection. In these paddocks, hay production removed herbage to a lower stubble height, and less residual stem and mature material remained. The resulting canopy consisted of mostly leaves making diet selection easier (Minson, 1990).

In September 2004, ADG of steers increased in all treatments when nutritive value improved. An application of N in August increased forage CP on all treatments, and fiber concentrations were low on these paddocks. In September 2005, ADG of steers grazing E+ decreased while those on E- and Q were similar (Table 3-3). Increased forage alkaloid levels due to drought conditions may have increased the effects of fescue toxicosis during this time decreasing animal performance.

Temperature

There was an effect of treatment on rectal temperature during the 2005 season and across the seasons ($P<0.05$). Steers grazing E+ had elevated temperatures compared to those grazing E- and Q (Table 3-3). These differences among treatments are similar to data reported by Parish et al. (2003b) and Nihsen et al. (2004) on cattle grazing nonergot alkaloid-producing endophyte-infected, E-, and E+ tall fescues. Alkaloids produced by E+ tall fescue have shown to have vasoconstrictive properties in the peripheral vasculature of cattle (Oliver et al., 1993). This can decrease the ability to dissipate heat and thus exacerbate heat stress when ambient temperatures and relative humidity are elevated (Browning and Leite-Browning, 1997).

There were no differences in tympanic temperature among treatments (E+, Q, and L) during any of the 5 d-periods that it was recorded. There was an effect of time on tympanic temperature ($P < 0.001$). The daily average maximum temperature between 1700 and 1800 was higher ($P < 0.05$) than the average minimum temperature between 0700 and 0800 in all four periods. In late spring, steer tympanic temperatures fluctuated less over the 24-hr period (Figure 3-1). During this period, THI indicated mild stress for 8 h during the day (Figure 3-2). Maximum daily temperatures of steers grazing E+ (39.9ºC) in July were numerically higher ($P = 0.37$) than in steers grazing L and Q (39.7

and 39.7ºC, respectively; Figure 3-3). Based on THI steers spent the longest time under conditions of mild stress (mean of 4 h/d) and under medium stress (mean of 8 h/d) during July (Figure 3-4). In August, fluctuations in steers' body temperatures resembled those in July although daily maximum temperatures only reached 39.5, 39.5, and 39.2 ºC on steers grazing E+, L, and Q, respectively (Figure 3-5). During this period animals spent less time in medium stress conditions (6 hr/d), compared to July (Figure 3-6), and average solar radiation during daylight hours was lower in August (3.99 kW/m^2) compared to July (4.55 kW/m^2). Keren and Olson (2006) developed a model of thermal balance in grazing cattle and determined that solar radiation contributes strongly to the thermal balance of cattle which may explain the lower temperatures in August compared to July. In September, maximum temperatures registered in steers ranged from 39.4 to 39.7 ºC. Also, steers showed less oscillation in temperature compared to the previous two periods (Figure 3-7). In July steers experienced 9 h/d of mild stress based on THI (Figure 3-8). Steers were only exposed to medium stress during two periods of temperature recordings. Al-Hairdary et al. (2001) reported that heifers that were implanted with telemetric temperature transmitters and consuming an E+ diet during continuous heat stress (31 ºC, 50% relative humidity, and 26.5 THI) had increased core body temperature compared to heifers consuming an E- diet. In the current experiment, nighttime temperatures fell below 20 ºC for at least 10 h during each temperature recording period. This allowed all steers to dissipate excess body heat gained during daytime heat stress unlike in continuous heat stress conditions described by Al-Hairdary et al. (2001). Although no differences were detected in steers' body temperature, steers grazing E+ exhibited signs of fescue toxicosis (intolerance to heat, idling, lounging in

water) during times of medium heat stress (Figure 3-9). The time spent lounging in water may have allowed steers grazing E+ to dissipate some heat during the hottest part of the may possibly contributing to the similar temperatures between these steers and those grazing Q and L. Furthermore, during this grazing season, steers on E+ increased ($P < 0.05$) time idling (371 min/d) compared to steers on Q and L (278 and 224 min/d, respectively; Boland, 2005). These data suggest that animals consuming an E+ diet are more sensitive to increased body temperature compared to animals consuming an alkaloid free diet.

Alkaloids

In 2004 and 2005, ergovaline and lysergic acid amide (LSA) were not detectable in forage samples of L, Q, and E- and in Q and E-, respectively (data not shown). In 2004, ergovaline levels in E+ forage were highest in late spring (562 μg/kg; Figure 3-10). This is consistent with the findings of Rottinghaus (1991) in which ergovaline concentration of forage was increased due to the presence of seedheads. Due to the asexual nature of the endophyte, as fescue enters a reproductive stage in late spring, the endophyte and ergovaline concentrate in the seedhead (Siegel et al., 1987). Unlike ergovaline, LSA levels were lowest during this time (60 μg/kg). After seedheads were removed, ergovaline levels decreased (281 μg/kg) while LSA increased (217 μg/kg). Levels of both ergovaline and LSA remained similar in late summer (244 and 235 μg/kg, respectively). In 2005, forage alkaloids were determined after seedheads were removed. In late spring (2005), ergovaline (300 μg/kg) was lower and LSA was higher (280 μg/kg) compared to late spring of 2004 (Figure 3-11). Ergovaline and LSA decreased slightly in mid-summer (247 and 160 μg/kg, respectively) before increasing in late summer/early fall (330 and 424 μg/kg, respectively). The presence of the endophyte in tall fescue

increases plant tolerance to adverse ambient stresses including drought (Schardl et al., 2004). Ergovaline concentrations in tall fescue plants are increased under drought conditions (Arechavaleta et al., 1992). Therefore, dry conditions prior to sampling in September of 2005 may explain the increase in forage alkaloid levels.

Little literature is available related to LSA levels in forage. Lysergic acid amide is a chemical analog of lysergic acid that has been reported to occur naturally in E+ tall fescue (Petroski and Powell, 1991). In humans, LSA is not hallucinogenic, but has shown to cause hypersalivation, dizziness, and diarrhea (Fanchamps, 1978). Sleepygrass (*Stipa robusta*), a grass known to have high levels of LSA, produced sedation when consumed by horses (Powell et al., 1991). Oliver (1993) reported LSA has vasoconstrictor activity and acts as a partial agonist at adrenergic receptors and antagonist at serotonergic receptors in bovine lateral saphenous vein and dorsal metatarsal artery. However, little research has been conducted trying to relate this compound with fescue toxicosis. The data from the current research suggest, although not specifically measured, LSA levels in reproductive tillers are low compared to other plant parts. Low levels were detected in whole plant samples when these structures were present compared to high levels in forage regrowth. Also, in drought conditions, LSA levels increased more than ergovaline.

In 2004 no LSA was detectable in ruminal fluid of steers prior to the beginning of the grazing season (Figure 3-12). Prior to the experiment, all steers were backgrounded on an alkaloid-free diet for 60 d. During early spring, LSA was detected (18.5 μg/L), decreased to a minimum in July (12.0 μg/L) and increased in late summer (16.8 μg/L). In 2005, LSA was detectable in samples of ruminal fluid taken before the beginning of

the experiment (5.3 µg/L; Figure 3-13). The pretreatment diet of these steers is unknown; but it is likely that some animals grazed E+ tall fescue prior to their sale. In late spring, ruminal fluid LSA concentration was 2.5 times higher than samples at a similar time in 2004 (44.5 µg/L), decreased in mid summer (12.0 µg/L) and increased slightly in late summer (16.8 µg/L). Ruminal fluid LSA concentration was higher during late spring/early summer of 2005 compared to 2004, although forage alkaloid levels were similar. Previous research has pointed to ergovaline as the toxic alkaloid causing fescue toxicosis due to its high recovery from E+ tall fescue (Agee and Hill, 1994). However, due to the site and higher potential of transport, Hill et al. (2001) suggested that a simple ergot alkaloid, and not an ergopeptine alkaloid, is responsible for fescue toxicosis. Stuedemann et al. (1998) demonstrated that urinary appearance of alkaloids occurred within 24 h of steers consuming an E+ diet. Excretion of ergot alkaloids is dependent on molecular weight (Eckert et al., 1978). Except for lysergic acid diethylamide, all alkaloids that are less than 350 Da are excreted in urine, those between 350 and 450 Da are excreted in urine or feces, and those bigger than 450 Da are excreted in the bile (Eckert et al., 1978). Therefore due to the rate at which alkaloids appear in urine, Stuedemann et al. (1998) suggested that alkaloids released from the forage by ruminal microorganisms are rapidly transformed or metabolized to ergoline alkaloids or to biotransformed ergopeptine. No literature is available on the appearance of LSA in ruminal fluid of animals grazing E+ tall fescue. Another simple ergoline alkaloid, lysergic acid was quantified in different matrices: forage, ruminal fluid, urine, and feces (Lodge-Ivey et al., 2006) of steers consuming E+ tall fescue straw with 400 µg/kg ergovaline. These authors reported that straw, ruminal fluid, urine, and feces contained

24.2, 13.3, 26.3, and 20.6 μg/kg, respectively. These findings along with those of the current research suggest that these simpler alkaloids are present in the rumen as metabolites of larger alkaloids and/or in their original form from plant material and are available for transport across gastric tissue.

CONCLUSIONS

Average daily gains of steers grazing Q and L were higher than for steers grazing E+ and similar to those of steers grazing E-. However, these forages may not be the optimal choice to complement or replace E+ tall fescue in grazing systems due to the lower ADG of Q compared to E- and the increased management input required to achieve this production level with L. Although steers were not exposed to prolonged heat stress, and despite similar body temperatures, those grazing E+ showed intolerance to heat compared to animals grazing Q and L. This is the first report of the appearance of LSA in the ruminal fluid of animals grazing endophyte-infected tall fescue. This appearance of LSA in ruminal fluid suggests that this compound may contribute to fescue toxicosis. Biotransformation and bioavailability of this compound in the rumen needs further investigation to determine possible strategies to decrease the occurrence of fescue toxicosis in grazing livestock.

TABLES AND FIGURES

Table 3-1. Monthly and 11-yr average rainfall (mm) during the 2004 and 2005 experimental period

Month	2004	2005	11 yr Avg
April	88	51	70
May	94	43	100
June	138	43	112
July	74	106	112
August	62	66	84
September	122	4	88
October	59	63	45
Total	636	376	611

Table 3-2. Least squares means of nutritive value and herbage mass (HM)[1] seasonal averages for Kentucky-31 endophyte infected, endophye free, and Q4508-AR542 non-ergot alkaloid-producing endophyte infected tall fescues, and Lakota prairie grass

	Treatment[2]				
Item	E-	Q	E+	L	SE
NDF, % of DM					
2004	59.0	59.2	57.9	57.8	0.7
2005	61.2[b]	61.7[c]	60.4[a]		0.2
Avg	60.3	60.8	59.1		0.4
ADF, % of DM					
2004	32.1[ab]	32.1[ab]	31.5[a]	32.8[b]	0.3
2005	31.1	31.3	30.3		0.4
Avg	31.6	31.8	30.8		0.3
CP, % of DM					
2004	15.6	15.7	15.9	16.7	0.5
2005	13.3	12.6	13.3		0.2
Avg	14.5	14.6	14.6		0.4
HM, kg/ha					
2004	2880[ab]	3090[b]	3150[b]	2530[a]	180
2005	3270	3570	3760		170
Avg	3070[a]	3360[b]	3420[b]		180

[1] There was no treatment x year for NDF ($P = 0.80$), ADF ($P = 0.96$), CP ($P = 0.74$), and HM ($P = 0.84$)

[2] Treatments: E+ = Kentucky 31 endophyte infected tall fescue; E- = endophye free tall fescue; Q = Q4508-AR542 non-ergot alkaloid-producing endophyte infected tall fescue; L = Lakota prairie grass.

[abc]Within a row, means without a common superscript letter differ ($P < 0.05$).

Table 3-3. Least squares means of seasonal average daily gain (ADG) and rectal temperatures (ºC)[1] for steers grazing Kentucky-31 endophyte infected and endophye free , Q4508-AR542 non-ergot alkaloid-producing endophyte infected tall fescues, and Lakota prairie grass

	Treatment				
Item	E-	Q	E+	L	SE
ADG, kg/d					
2004	0.54[b]	0.42[b]	0.22[a]	0.58[b]	0.06
2005	0.54[b]	0.49[b]	0.37[a]		0.02
Avg	0.54[b]	0.45[b]	0.30[a]		0.03
Rectal Temp, ºC					
2004	39.6	39.3	39.5	39.6	0.08
2005	39.4[a]	39.3[a]	39.9[b]		0.08
Avg	39.5[b]	39.3[a]	39.7[c]		0.02

[1]There was no treatment x year for ADG ($P = 0.69$) or rectal temperature ($P = 0.09$).
[2] Treatments: E+ = Kentucky 31 endophyte infected tall fescue; E- = endophye free tall fescue; Q = Q4508-AR542 non-ergot alkaloid-producing endophyte infected tall fescue; L = Lakota prairie grass.
[abc]Within a row, means without a common superscript letter differ ($P < 0.05$).

Table 3-4. Least squares means for period average daily gain (ADG) of steers grazing Kentucky 31 endophyte infected and endophye free, Q4508-AR542 non-ergot alkaloid-producing endophyte infected tall fescues, and Lakota prairie grass pastures

	Treatment[1]				
Year/Month	E-	Q	E+	L	SE
2004					
May	0.71[ab]	0.81[a]	0.35[b]	0.85[a]	0.13
June	0.00	0.01	0.02	0.07	0.06
July	0.62[ab]	0.63[ab]	0.24[b]	0.80[a]	0.14
August	0.39	0.22	0.16	0.27	0.10
September	0.87	0.70	0.51	0.92	0.15
2005					
May	1.31	1.51	0.93		0.33
June	0.52	0.42	0.33		0.62
July	0.15	0.00	0.00		0.16
August	0.70	0.62	0.59		0.05
September	0.48	0.57	0.23		0.12

[1] Treatments: E+ = Kentucky 31 endophyte infected tall fescue; E- = endophye free tall fescue; Q = Q4508-AR542 nonergot alkaloid -producing endophyte infected tall fescue; L = Lakota prairie grass.

[ab]Row means within month with different superscripts differ, $P < 0.05$.

Table 3-5. Least squares means of period nutritive value and herbage mass (HM) for Kentucky-31 endophyte infected, endophye free, and Q4508-AR542 non-ergot alkaloid-producing endophyte infected tall fescues, and Lakota prairie grass in 2004

Date/Treatment[1]	NDF, % of DM	ADF, % of DM	CP, % of DM	HM, kg/ha
May 5				
E-	48.0	25.3	24.2[ab]	3040[ab]
Q	49.8	26.6	22.5[bc]	2960[ab]
E+	47.2	25.0	24.7[a]	3920[a]
L	52.4	27.4	21.5[c]	2180[b]
SE	1.5	1.0	0.5	510
May 27				
E-	68.4	40.4	8.6	3840[a]
Q	68.3	39.5	9.4	3650[a]
E+	69.7	41.1	9.0	3870[a]
L	70.9	40.6	9.3	2360[b]
SE	0.8	0.9	0.6	400
June 24				
E-	61.0	34.0	14.0	3330
Q	61.0	34.4	16.1	3440
E+	58.5	32.8	15.6	3070
L	54.1	31.2	18.7	2590
SE	2.3	1.6	1.6	570
July 22				
E-	55.9[b]	29.9	13.7	1800
Q	56.3[b]	29.7	14.4	2240
E+	55.4[b]	28.4	14.1	2340
L	60.3[a]	32.7	14.9	2350
SE	0.9	1.1	1.0	360
August 20				
E-	66.4[a]	35.5	12.7[ab]	3700
Q	64.8[ab]	33.8	14.0[a]	4020
E+	62.1[b]	34.1	11.2[b]	3710
L	62.2[b]	36.3	13.7[a]	3620
SE	0.9	0.5	0.5	370
September 17				
E-	56.6[ab]	27.3	20.7[a]	1670
Q	58.5[a]	28.6	17.8[b]	2350
E+	56.5[b]	27.5	21.1[a]	1970
L	55.2[b]	28.7	22.2[a]	1940
SE	0.9	0.5	0.8	300

[1] Treatments: E+ = Kentucky 31 endophyte infected tall fescue; E- = endophye free tall fescue; Q = Q4508-AR542 non-ergot alkaloid-producing endophyte infected tall fescue; L = Lakota prairie grass.

[abc]Column means within sampling date with different superscripts differ, $P < 0.05$.

Table 3-6. Least squares means of period nutritive value, herbage mass (HM), and herbage allowance (HA) for Kentucky-31 endophyte infected, endophye free, and Q4508-AR542 non-ergot alkaloid-producing endophyte infected tall fescues in 2005

Date/Treatment[1]	NDF, % of DM	ADF, % of DM	CP, % of DM	HM, kg/ha
May 3				
E-	47.7[c]	22.6[a]	17.1[a]	2510
Q	52.5[a]	25.2[a]	15.1[b]	2560
E+	49.9[b]	23.6[ab]	15.9[b]	3440
SE	0.5	0.5	0.3	400
May 31				
E-	69.8	36.1	10.6	4320
Q	68.7	33.8	10.6	4760
E+	62.2	32.2	11.7	4590
SE	2.4	01.6	1.0	570
June 28				
E-	64.0[a]	33.2	11.7	3540
Q	65.5[a]	33.6	11.1	3720
E+	62.0[b]	32.5	12.1	4410
SE	0.6	0.4	0.4	380
July 26				
E-	64.6	33.8	12.1	2920
Q	69.0	33.8	11.6	2970
E+	62.8	32.6	12.2	3270
SE	3.6	0.9	0.4	310
August 23				
E-	63.4	31.6	13.6	4120
Q	63.5	31.8	13.9	3880
E+	62.9	31.7	13.3	3900
SE	0.6	0.5	0.4	270
September 20				
E-	57.6	29.2	14.8	2220
Q	59.2	30.1	13.5	3530
E+	56.8	28.7	14.3	3260
SE	0.8	0.5	0.5	540

[1] Treatments: E+ = Kentucky 31 endophyte infected tall fescue; E- = endophye free tall fescue; Q = Q4508-AR542 non-ergot alkaloid-producing endophyte infected tall fescue.
[abc]Column means within sampling date with different superscripts differ, $P < 0.05$.

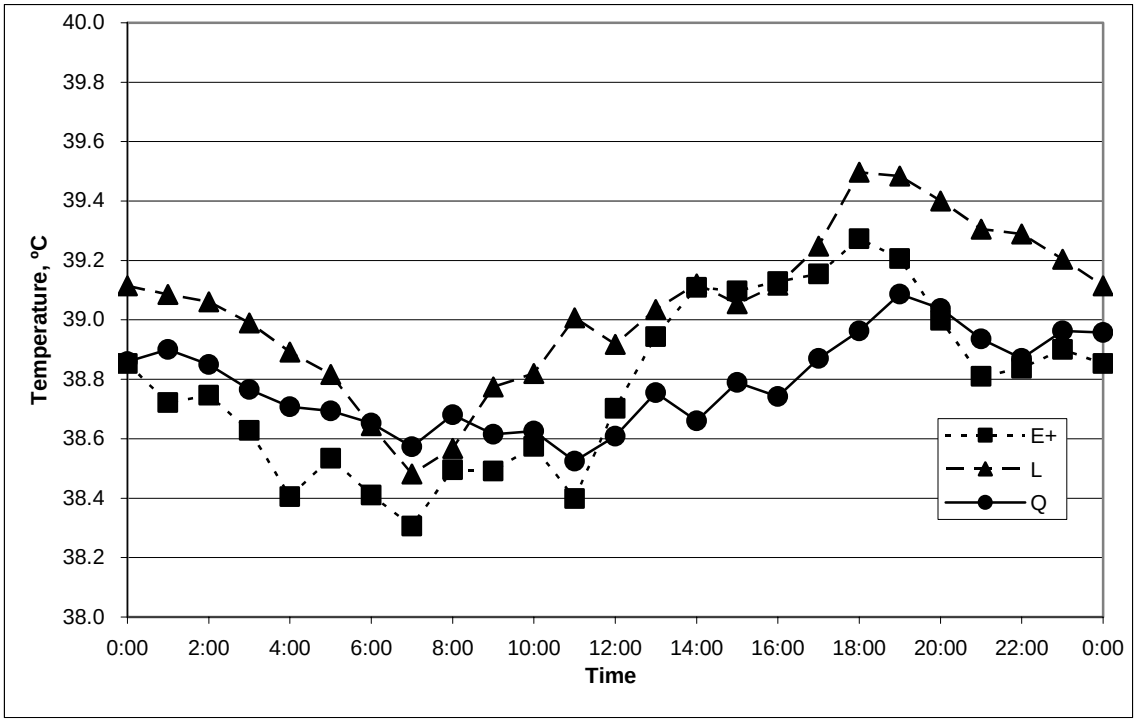


Figure 3-1. Average hourly diurnal body temperature of steers grazing Kentucky 31 endophyte infected (E+) and Q4508-AR542 non-ergot alkaloid-producing endophyte endophyte infected (Q) tall fescues and 'Lakota' prairie grass (L) pastures over a 5-d period (May 30 to June 4, 2004).

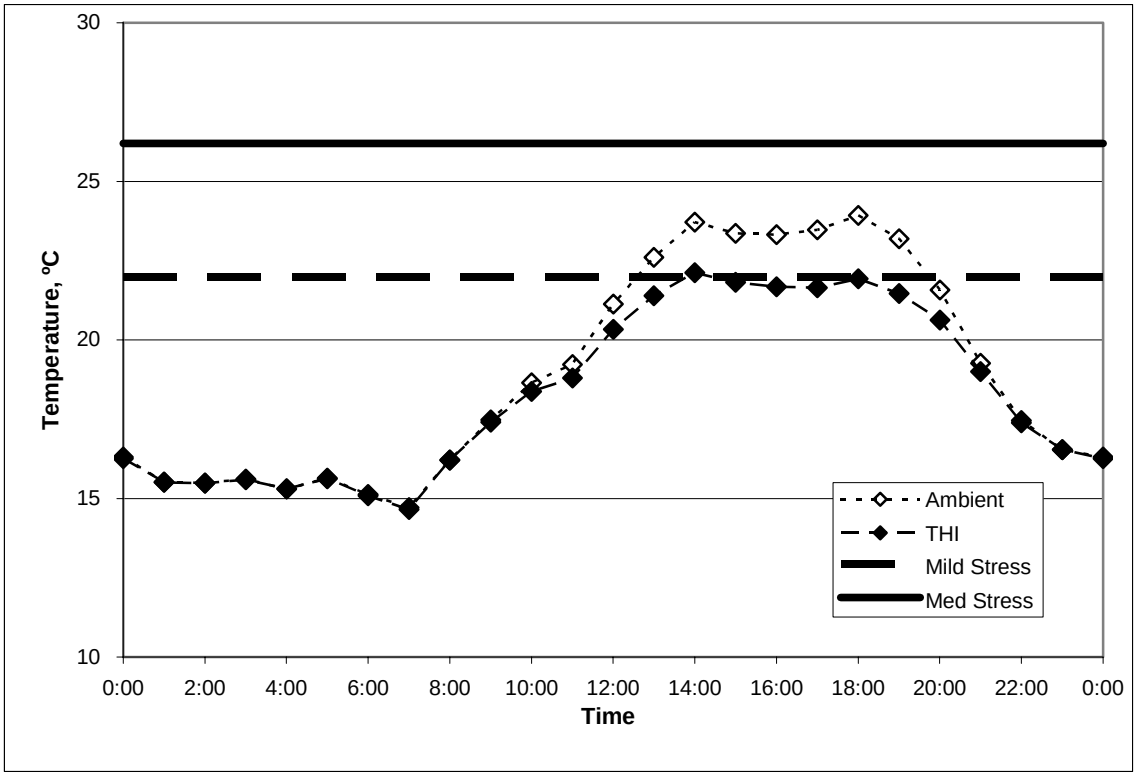


Figure 3-2. Average ambient temperature and temperature humidity index (THI) for May 30 to June 4, 2004.

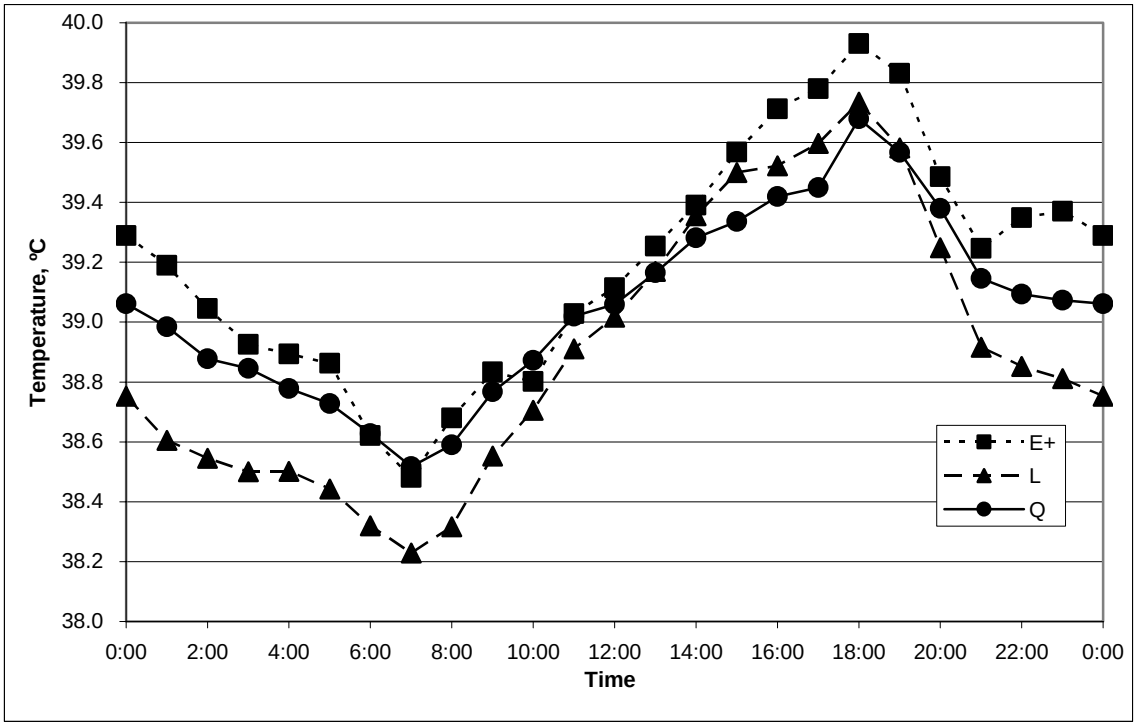


Figure 3-3. Average hourly diurnal body temperature of steers grazing Kentucky 31 endophyte infected (E+) and Q4508-AR542 non-ergot alkaloid-producing endophyte endophyte infected (Q) tall fescues and 'Lakota' prairie grass (L) pastures over a 5-d period (July 6 to July 11, 2004).

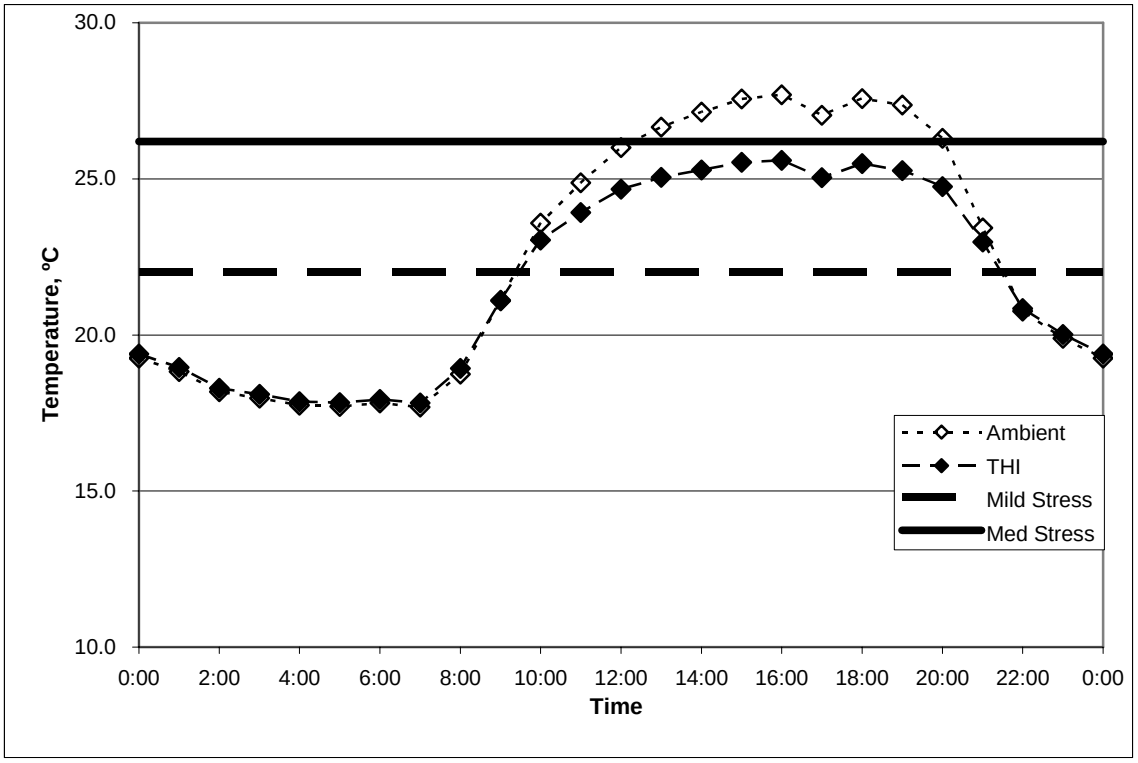


Figure 3-4. Average ambient temperature and temperature humidity index (THI) for July 6 to July 11, 2004.

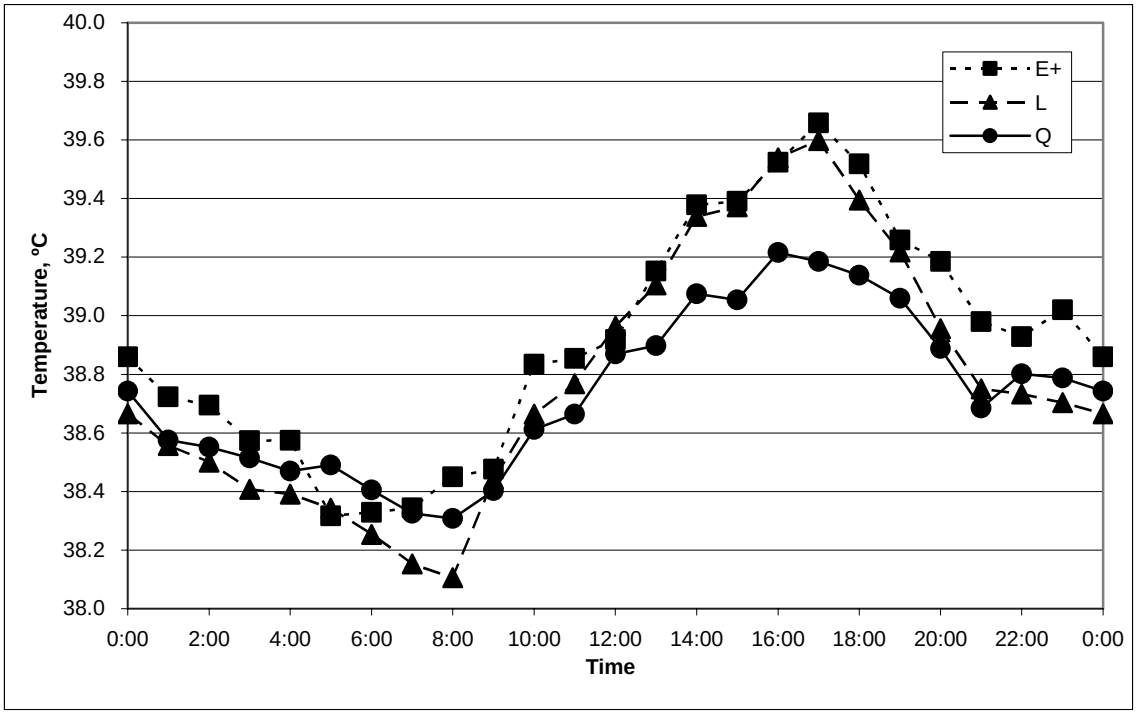


Figure 3-5. Average hourly diurnal body temperature of steers grazing Kentucky 31 endophyte infected (E+) and Q4508-AR542 non-ergot alkaloid-producing endophyte endophyte infected (Q) tall fescues and 'Lakota' prairie grass (L) pastures over a 5-d period (August 1 to August 6, 2004).

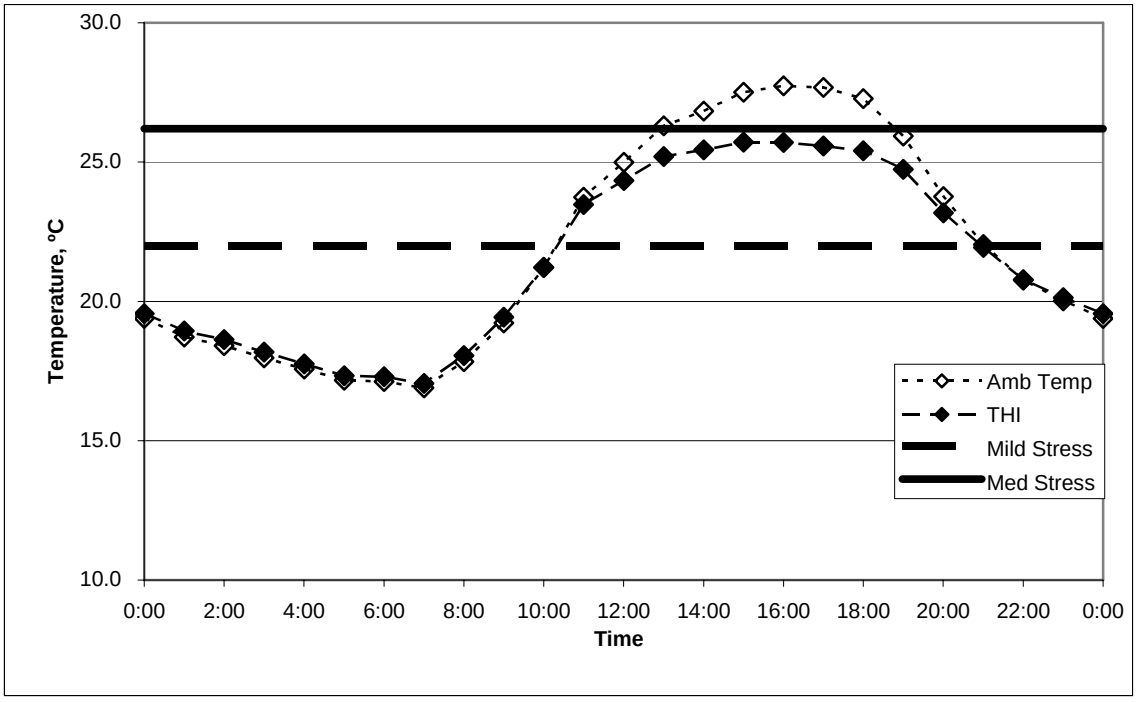


Figure 3-6. Average ambient temperature and temperature humidity index (THI) for August 1 to August 6, 2004.

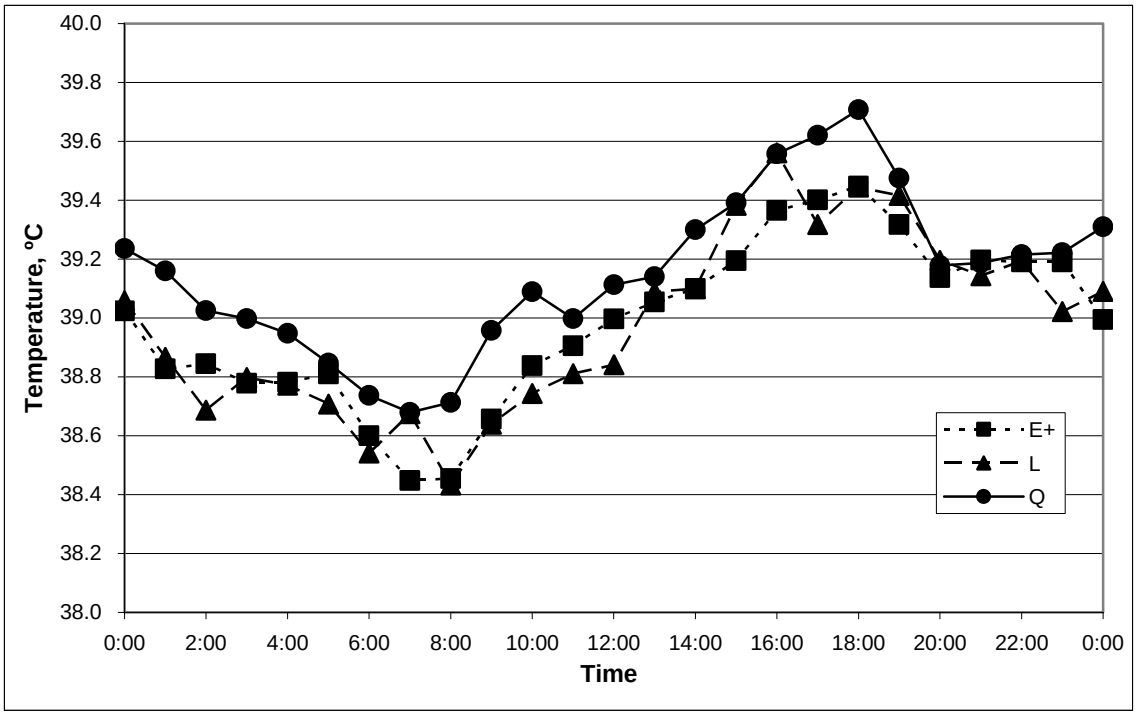


Figure 3-7. Average hourly diurnal body temperature of steers grazing Kentucky 31 endophyte infected (E+) and Q4508-AR542 non-ergot alkaloid-producing endophyte endophyte infected (Q) tall fescues and 'Lakota' prairie grass (L) pastures over a 5-d period (August 31 to September 5, 2004).

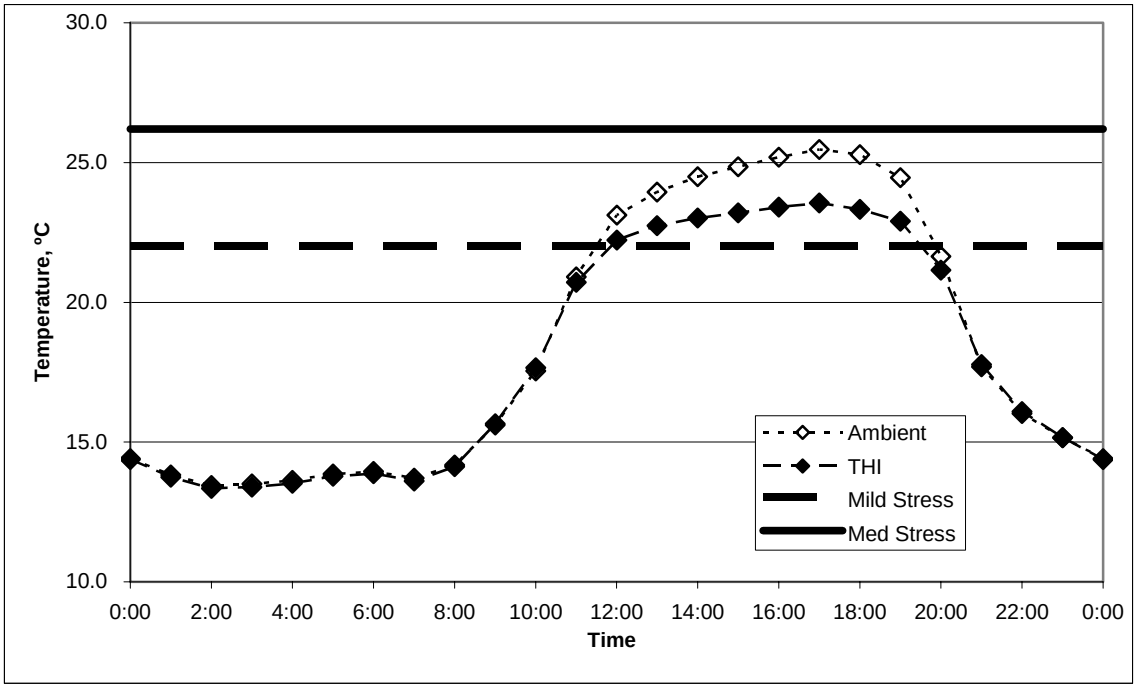


Figure 3-8. Average ambient temperature and temperature humidity index (THI) for August 31 to September 5, 2004.

Figure 3-9. Picture taken on July 11, 2004 in mid-afternoon, of E+ steers (in foreground) suffering heat stress due to fescue toxicosis, cooling themselves in water they splashed out of waterers (shown to the right). In the background, steers grazing E- are shown grazing and not suffering from heat stress (Picture courtesy of H.T. Boland).

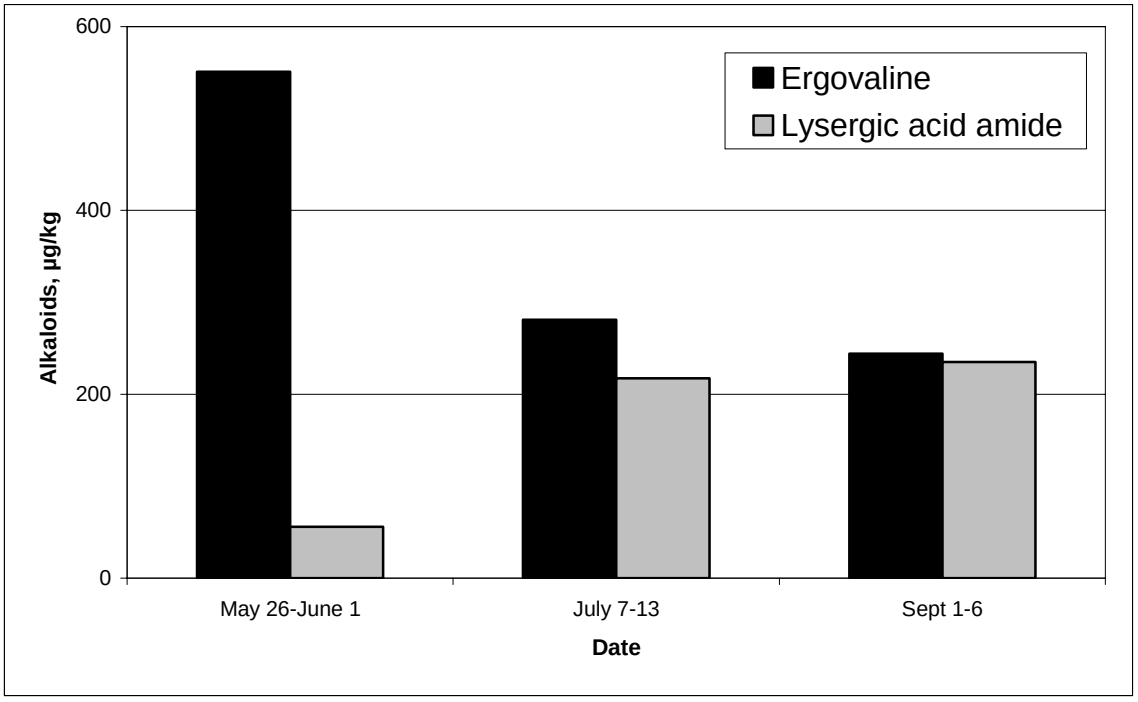


Figure 3-10. Ergovaline and lysergic acid amide levels of Kentucky 31 E+ pastures during 2004.

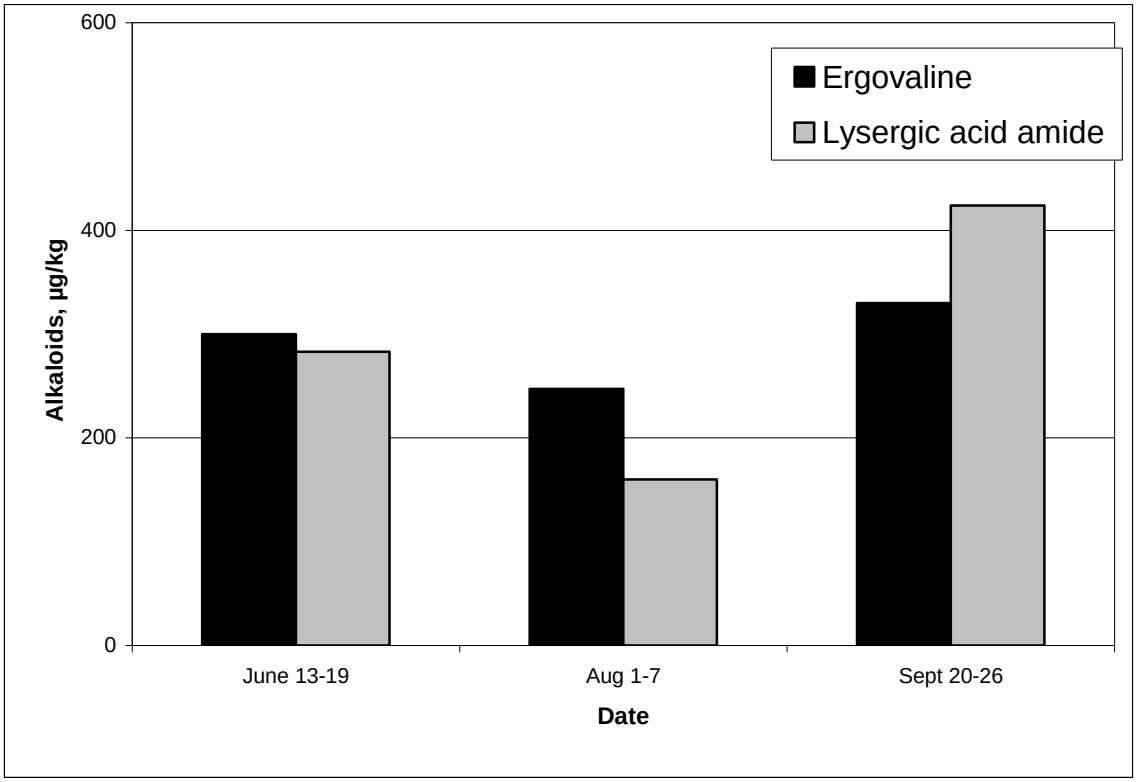


Figure 3-11. Ergovaline and lysergic acid amide levels of Kentucky 31 E+ pastures during 2005.

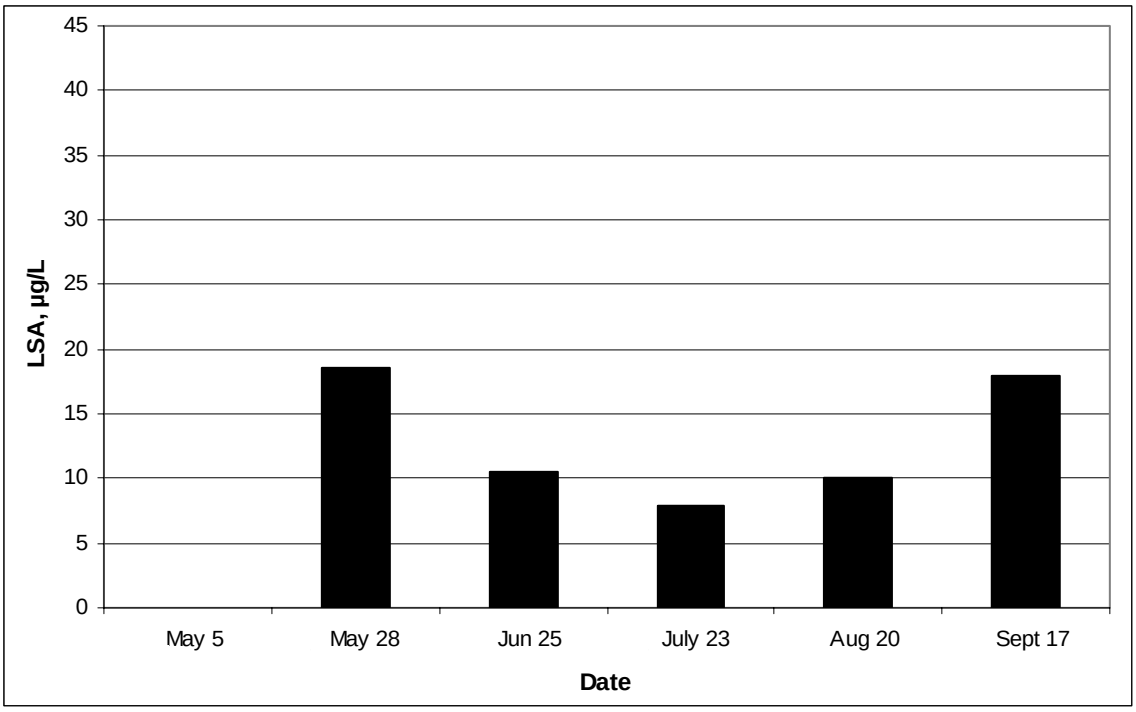


Figure 3-12. Lysergic acid amide (LSA) appearance in ruminal fluid of steers grazing Kentucky 31 E+ pastures during 2004.

.

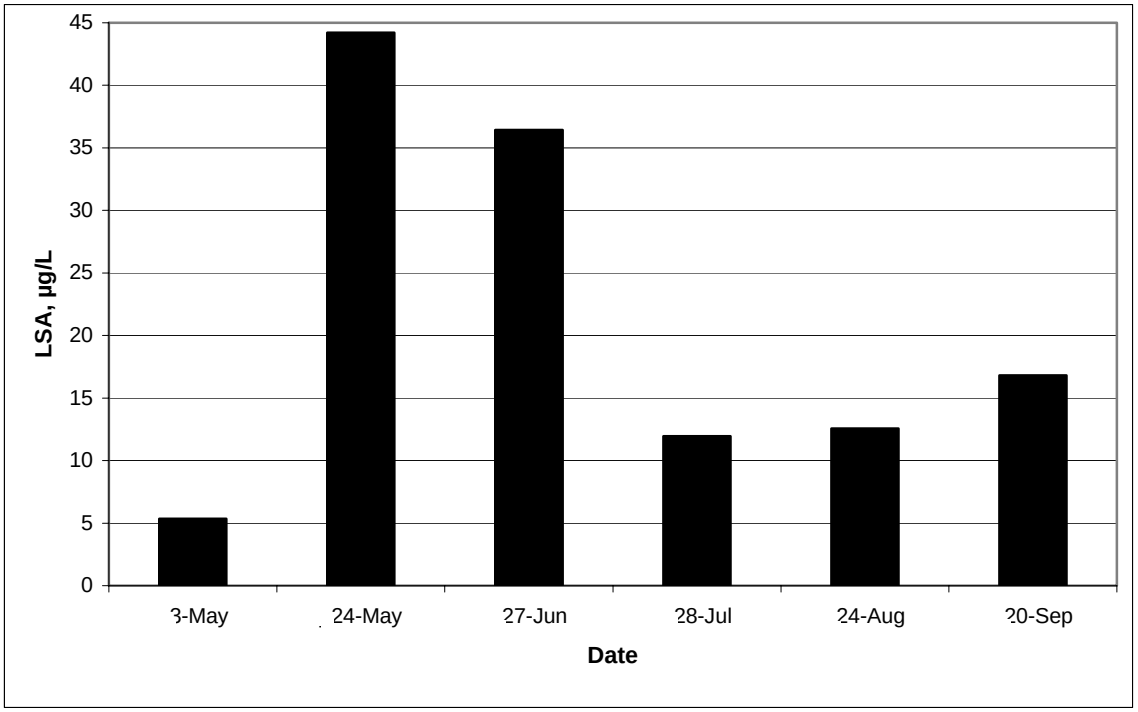


Figure 3-13. Lysergic acid amide (LSA) appearance in ruminal fluid of steers grazing Kentucky 31 E+ pastures during 2005.

LITERATURE CITED

Agee, C. S., and N. S. Hill. 1994. Ergovaline variability on *Acremonium* infected tall fescue due to environment and plant genotype. Crop Sci. 34: 221-226.

Al-Haidary, A., D. E. Spiers, G. E. Rottinghaus, G. B. Garner, and M. R. Ellersieck. 2001. Thermoregulatory ability of beef heifers following intake of endophyte-infected tall fescue during controlled heat challenge. J. Anim. Sci. 79: 1780-1788.

Allen, V. G., J. P. Fontenot, and R. A. Brock. 2000. Forage systems for production of stocker steers in the upper south. J. Anim Sci. 78: 1973-1982.

AOAC. 2000. Official Methods of Analysis (17th Ed.). Association of Official Analytical Chemists, Washington, DC.

Arechavaleta, M., C. W. Bacon, R. D. Plattner, C. S. Hoveland, and D. E. Radcliffe. 1992. Accumulation of Ergopeptide Alkaloids in Symbiotic Tall Fescue Grown under Deficits of Soil Water and Nitrogen Fertilizer. Appl. Environ. Microbiol. 58: 857-861.

Bacon, C. W., and M. R. Siegel. 1988. Endophyte parasitism of tall fescue. J. Prod. Agric. 1: 45-55.

Bouton, J. H., G. C. M. Latch, N. S. Hill, C. S. Hoveland, M. A. McCann, R. H. Watson, J. A. Parish, L. L. Hawkins, and F. N. Thompson. 2002. Reinfection of tall fescue cultivars with non-ergot alkaloid-producing endophytes. Agron. J. 94: 567-574.

Browning, R., Jr., and M. L. Leite-Browning. 1997. Effect of ergotamine and ergonovine on thermal regulation and cardiovascular function in cattle. J. Anim. Sci. 75: 176-181.

Dubbs, T. M., E. S. Vanzant, S. E. Kitts, R. F. Bapst, B. G. Fieser, and C. M. Howlett. 2003. Characterization of season and sampling method effects on measurement of forage quality in fescue-based pastures. J. Anim Sci. 81: 1308-1315.

Eckert, H., J. R. Kiechel, J. Rosenthaler, R. Schmidt, and E. Schreier. 1978. Biopharmaceutical aspects: Analytical methods, pharmacokinetics, metabolism, and bioavailability. In: B. Berde and H. O. Schild (eds.) Ergot Alkaloids and Related Compounds. p 719-803. Springer-Verlag, New York.

Fanchamps, A. 1978. Some compounds with hallucinogenic activity. In: B. Berde and H. O. Schild (eds.) Ergot Alkaloids and Related Compounds. p 567-614. Springer-Verlag, New York.

Hemken, R. W., J. A. Boling, L. S. Bull, R. H. Hatton, R. C. Buckner, and L. P. Bush. 1981. Interactions of environmental temperature and anti-quality factors on the severity of summer fescue toxicosis. J. Anim. Sci. 52: 710.

Hiatt, E. E. r., N. S. Hill, J. H. Bouton, and C. W. Mims. 1997. Monoclonal antibodies for detection of *Neotyphodium coenophialum*. Crop Sci. 37: 1265-1269.

Hill, N. S., F. N. Thompson, J. A. Stuedemann, G. W. Rottinghaus, H. J. Ju, D. L. Dawe, and E. E. r. Hiatt. 2001. Ergot alkaloid transport across ruminant gastric tissues. J. Anim. Sci. 79: 542-549.

Hoveland, C. S. 1993. Importance and economic significance of the Acremonium endophytes to performance of animals and grass plant. In: R. Joost and S. Quisenberry (eds.) Acremonium/Grass Interactions. p 3-12. Elsevier Science, Amsterdam, The Netherlands.

Hoveland, C. S., R. L. Haaland, C. D. Berry, J. F. Pedersen, S. P. Schmidt, and R. R. Harris. 1982. Triumph, a new winter-productive tall fescue variety. Alabama Agric. Exp. Bull. 260.

Keren, E. N., and B. E. Olson. 2006. Thermal balance of cattle grazing winter range: Model application. J. Anim. Sci. 84: 1238-1247.

Klopfenstein, T. J., D. J. Jordan, I. Rush, and T. Milton. 1999. Predicting amount of compensatory gain. Nebraska Beef Cattle Report. MP 71-A:: 26-28. Lincoln, NE.

Lodge-Ivey, S. L., K. Walker, T. Fleischmann, J. E. True, and A. M. Craig. 2006. Detection of lysergic acid in ruminal fluid, urine, and in endophyte-infected tall fescue using high-performance liquid chromatography. J. Vet. Diagn. Invest. 18: 369-374.

Lowe, K. F., T. M. Bowdler, N. D. Casey, and R. J. Moss. 1999. Performance of temperate perennial pastures in the Australian subtropics. 1. Yield, persistence and pasuture quality. Aust. J. Exp. Agric. 39: 663-676.

McCracken, B. A., L. J. Krysl, K. K. Park, D. W. Holcombe, and M. B. Judkins. 1993. Steers grazing endophyte-free tall fescue: Seasonal changes in nutrient quality, forage intake, digesta kinetics, ruminal fermentation, and serum hormones and metabolites. J. Anim. Sci. 71: 1588-1595.

Minson, D. J. 1990. Forage in Ruminant Nutrition. Academic Press, San Diego, CA.

Nelson, C. J., and L. E. Moser. 1995. Plant factors affecting forage quality. In: G. C. Fahey, Jr., M. Collins, D. R. Mertens and L. E. Moser (eds.) Forage Quality, Evaluation, and Utilization. p 115-154. Am. Soc. of Agron. Inc; Crop Sci. Soc. Am. Inc.; Soil Sci. Soc. Am. Inc., Madison, WI.

Nihsen, M. E., E. L. Piper, C. P. West, R. J. Crawford, Jr., T. M. Denard, Z. B. Johnson, C. A. Roberts, D. A. Spiers, and C. F. Rosenkrans, Jr. 2004. Growth rate and physiology of steers grazing tall fescue inoculated with novel endophytes. J. Anim Sci. 82: 878-883.

NOAA. 1976. Livestock weather stress. U.S. Dept. of Com., National Oceanic and Atmospheric Admin, National Weather Service Central Regioin. Regional Operations Manual Lett: C-31-76.

NRC. 2000. Nutrient Requirements of Beef Cattle. 7th ed. Natl. Acad Press, Washington, D.C.

Oliver, J. W., L. K. Abney, J. R. Strickland, and R. D. Linnabary. 1993. Vasoconstriction in bovine vasculature induced by the tall fescue alkaloid lysergamide. J. Anim. Sci. 71: 2708-2713.

Parish, J. A., M. A. McCann, R. H. Watson, C. S. Hoveland, L. L. Hawkins, N. S. Hill, and J. H. Bouton. 2003a. Use of nonergot alkaloid-producing endophytes for alleviating tall fescue toxicosis in sheep. J. Anim Sci. 81: 1316-1322.

Parish, J. A., M. A. McCann, R. H. Watson, N. N. Paiva, C. S. Hoveland, A. H. Parks, B. L. Upchurch, N. S. Hill, and J. H. Bouton. 2003b. Use of nonergot alkaloid-producing endophytes for alleviating tall fescue toxicosis in stocker cattle. J. Anim Sci. 81: 2856-2868.

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

Peters, C. W., K. N. Grigsby, C. G. Aldrich, J. A. Paterson, R. J. Lipsey, M. S. Kerley, and G. B. Garner. 1992. Performance, forage utilization, and ergovaline consumption by beef cows grazing endophyte fungus-infected tall fescue, endophyte fungus-free tall fescue, or orchardgrass pastures. J. Anim. Sci. 70: 1550-1561.

Petroski, R. J., and R. G. Powell. 1991. Preperative separation of complex alkaloid mixture by high-speed countercurrent chromatography. In: P. A. Hedin (ed.)

Naturally Ocurring Pest Bioregulators. p 436-434. Am. Chem. Soc., Washington, DC.

Porter, J. K. 1995. Analysis of endophyte toxins: fescue and other grasses toxic to livestock. J. Anim Sci. 73: 871-880.

Powell, R. G., R. J. Petroski, and K. Clay. 1991. Alkaloids of *Stipa robusta* ("sleepy grass") infected with an *Acremonium* endophyte as compared to the alkaloid of tall fescue. In: Proc. Tall Fescue Toxicosis Workshop, SERA-IEG-37, Atlanta, GA. p 37-49.

Rottinghaus, G. E., G. B. Garner, C. N. Cornell, and J. L. Ellis. 1991. HPLC method for quantitating ergovaline in endophyte infested tall fescue: Seasonal variation of ergovaline levels in stems with leaf sheaths, leaf blades, and seed heads. J. Agric. Food Chem. 39: 112-115.

Rotz, J. D. 2006. Comparison of techniques for estimating pasture herbage mass and productive ground cover for Lakota prairie grass, Kentucky 31 endophyte free tall fescue, Kentucky 31 endophyte infected tall fescue and Quantum 542 tall fescue grazed by stocker cattle, M.S. Thesis, Virginia Tech, Blacksburg.

Schardl, C. L., A. Leuchtmann, and M. J. Spiering. 2004. Symbioses of grasses with seedborne fungal endophytes. Annual Review of Plant Biology 55: 315-340.

Siegel, M. R., G. C. M. Latch, and M. C. Johnson. 1987. Fungal endophytes of grasses. Annu. Rev. Phytopathol. 25: 293-315.

Stuedemann, J. A., N. S. Hill, F. N. Thompson, R. A. Fayrer-Hosken, W. P. Hay, D. L. Dawe, D. H. Seman, and S. A. Martin. 1998. Urinary and biliary excretion of ergot alkaloids from steers that grazed endophyte-infected tall fescue. J. Anim Sci. 76: 2146-2154.

Stuedemann, J. A., and C. S. Hoveland. 1988. Fescue endophyte history and impact on animal agriculture. J. Prod. Agric. 1: 39-44.

VAES. 2006. College Farm Operations, College of Agriculture and Life Sciences, weather data. Available: http://www.vaes.vt.edu/colleges/kentland/weather/ Accessed January 28, 2006.

CHAPTER 4
INTAKE DETERMINATION OF STEERS GRAZING THREE FESCUE TYPES AND LAKOTA PRAIRIE GRASS USING THE ALKANE TECHNIQUE

ABSTRACT

Dry matter intake of grazing animals is considered the single most limiting factor affecting animal performance. Also, decreased DMI has been suggested to contribute to reduced performance of animals grazing tall fescue [*Schedonorus arundinaceus* (Schreb.) Dumort.] infected by the endophyte *Neotyphodium coenophialum*. The objective of this research was to use the alkane method (dosed alkane C_{32} and natural alkane C_{33}) to estimate DMI of steers grazing Kentucky-31 endophyte infected (E+) and endophyte free (E-) tall fescues, Q4508-AR542 non-ergot alkaloid-producing endophyte infected tall fescue (Q), and Lakota prairie grass (L; (*Bromus catharticus* Vahl.). All forages were evaluated in 2004 and only fescue types in 2005. Dry matter intake estimates did not differ ($P = 0.88$) when based on samples collected at 0800, 1700, or a composite of the two sampling times. Estimation of DMI using forage collected by hand to mimic the selectivity of the forage exerted by the grazing animal tended to be higher ($P = 0.06$) than DMI estimated from whole plant clipped samples. In 2004, DMI of steers grazing E- was higher ($P < 0.001$) than Q, E+, and L, while DMI of steers grazing Q and E+ were similar ($P > 0.25$). In 2005, DMI did not differ ($P = 0.23$) among fescue types. These results suggest that decreased DMI affects performance of steers grazing of E+, and lower intake of Q suggests that the fescue variety Q4508 may not be the optimal variety for the incorporation on non-ergot alkaloid-producing endophyte strains.

INTRODUCTION

Animal performance is a function of total DMI, DMD, and animal genetics. However, DMI and DMD are the most variable components in grazing systems. Therefore, DMI and DMD represent useful estimates of forage quality when actual animal performance cannot be measured (Sollenberger and Cherney, 1995). In a conventional digestion trial, both intake and fecal output can be measured to calculate total DMI and DMD. However, in grazing animals, this cannot be accomplished without affecting their grazing behavior (Lippke, 2002). Therefore, under grazing conditions, another method of measuring these parameters is needed. One alternative to total fecal collection is to estimate fecal output using markers. Markers are compounds considered internal or external to the feedstuff. They are characterized as being 1) unabsorbable, 2) not affected by or affect the animal or microbial digestive processes, 3) evenly distributed throughout and having the same passage rate as the digesta, and 4) analyzable with specific and sensitive methodology (Owens and Hanson, 1992). Several markers have been utilized, but it has been suggested that none of these markers fulfill all of the previous characteristics (Merchen, 1988).

Alkanes are long-chained saturated aliphatic hydrocarbons that are, although minor, a component ubiquitous to the cuticular wax of higher plants. Plant alkanes typically have an odd number of carbon atoms, with chain length varying from 21 to 37 carbon atoms (Mayes et al., 1986b). These compounds are easily analyzed by gas chromatography and the inertness of these compounds make them a potential marker in grazing situations (Mayes and Lamb, 1984).

Infection of tall fescue [*Schedonorus arundinaceus* (Schreb.) Dumort.] by an endophytic fungus (*Neotyphodium coenophialum*) has been associated with a disorder in

grazing animals referred to as fescue toxicosis caused by the consumption of ergot alkaloids (Porter and Thompson, 1992). Non-ergot alkaloid-producing endophyte strains have been developed to potentially eliminate the class of alkaloids associated with decreased animal performance while maintaining those compounds associated with improved plant persistence (Bouton et al., 2002). Lower DMI of animals grazing endophyte-infected tall fescue is considered a contributor to decreased animal performance compared to other fescue types (Parish et al., 2003b). Dry matter intake of fescue types is well documented utilizing chromic oxide as an external marker (Paterson et al., 1995; Judkins et al., 1997; Elizalde et al., 1998; Abaye et al., 2002; Parish et al., 2003b), but literature is limited comparing DMI of these grasses using alkanes as external markers (Lopez-Guerrero, 2005; Scaglia et al., 2005) and comparing new varieties of fescue utilizing non-ergot alkaloid-producing endophytes (Parish et al., 2003b).

The objectives of this research were to determine the DMI of steers grazing three fescue types and 'Lakota' prairie grass (*Bromus catharticus* Vahl.), to compare the effect of time that samples of fecal matter are obtained on DMI estimation and to evaluate different forage sampling methods for DMI estimation.

METHODS AND MATERIALS

Experimental site

This experiment was conducted at Virginia Tech's Kentland Farm located west of Blacksburg, VA (37°11' N, 80°35' W). Pasture and animal management are described in Chapter 3.

Treatments and design

Intake trials were conducted during the grazing seasons of 2004 and 2005. Treatments were defined as forage type and included wild-type endophyte infected

Kentucky 31 tall fescue (E+), endophyte free Kentucky 31 tall fescue (E-), non-ergot alkaloid-producing endophyte (strain AR542) infected Q4508 tall fescue (Q), and Lakota prairie grass (L). Prairie grass was not used in the 2005 season. Treatments were arranged in two replicates of a randomized complete block design with soil type as block and pasture as the experimental unit (n=8, 2004; n=6, 2005).

Sample collecting

Samples were collected during three periods throughout each grazing season (Table 4-1). During these periods, animals were dosed (d 0) with a controlled release device (CRD) fabricated to be used in large cattle (300-700 kg) as indicated by the manufacturer (Captec Ltd., Nufarm, Auckland, NZ). The CRD contained 7601 mg of the alkanes dotriacontane (C_{32}) and hexatriacontane (C_{36}). The release rate stated by the manufacturer for the CRD was 354.6 mg/d. Seven days were allowed for the marker concentration to reach equilibrium. From d 7 to 14, fecal samples were collected twice daily (0800 and 1700) from each dosed steer. Steers were observed until they defecated, at which point feces were collected with care taken to avoid contamination by foreign material. If a fecal sample was not obtained in the pasture, steer(s) was (were) moved to a working facility where grab samples were taken. Forage sampling began on d 5 and continued through d 12 of each period based on approximately 48 h retention time of forage material (McCracken et al., 1993). Forage samples were collected prior to or during the AM fecal collection by walking an "X" in the paddock and stopping every 10 steps to collect a sample. Samples collected in each paddock consisted of a whole plant sample (WP) and a hand-plucked sample (HP; harvesting approximately the top 8 cm of the canopy) to mimic the selectivity of the forage exerted by the grazing animal. Both

fecal and forage samples were placed on ice for transport to the laboratory where they were frozen at -20 °C until further analysis.

Laboratory procedures

Forage characteristics

Forage nutritive value and herbage mass was determined as described in Chapter 3. All samples were obtained prior to animals entering the paddock in which DMI was measured.

Alkane analysis

All samples described were handled and analyzed in duplicate. Forage samples were lyophilized in a FreeZone 12 L lyophilizer (Labconco, Co., Kansas City, MO) or a VirTis Genesis 25 EL lyophilizer (SP Industries, Inc., Gradiner, NY). The dried sample was ground to pass a 1-mm screen in a Wiley mill (Thomas Wiley, Laboratory Mill Model 4, Arthur H. Thomas Co. Philadelphia, PA) and then a 0.5 mm screen in a Tecator Cyclotec 1093 sample mill (Tecator, Hogänäs, Sweden). Fecal samples were thawed at room temperature for approximately 24 h. For each animal on each day, 20 g of fresh material were weighed out for both AM and PM samples into a beaker and covered with cheesecloth. Ten grams of both AM and PM samples for each animal on each day were placed in a beaker as a composite sample for the given day. Weighed fecal samples were then freeze dried and ground to pass a 0.5-mm screen in a Tecator Cyclotec 1093 cyclone mill (Tecator, Hogänäs, Sweden).

Freeze-dried, ground samples were analyzed for alkanes as described by Mayes et al. (1986). Briefly, 0.3 and 0.1 g of forage and feces, respectively, were placed along with 10 mg of internal standard (C_{34}, n-tetratriacontane) in 20-mL Pyrex screw cap culture tubes. The samples were saponified with 7 mL of a 10% ethanoic KOH solution

at 90 °C for 3 h in a water bath. Contents were vortexed every 30 min. After cooling, 7 mL of deionized water and 7 mL of heptane were added and the contents were vortexed for approximately 15 s. The organic extract was removed and applied to a filtration column made of 5 mL disposable pipettes containing silica gel with a glass wool stopper. The extract was eluted with approximately 10 mL of heptane into 20 mL scintillation vials. The eluent was then dehydrated in a N evaporator unit (Organonmation Associates, Inc. Berlin, MA, USA), and redissolved in 1 mL of heptane. An injection of 0.5 µL of each sample was applied to the capillary column (30 m long, 0.52 mm i.d., and 1.5 µm of fused silica film thickness; Restek Inc., Bellefonte, PA) of a gas chromatograph (Agilent Technologies 6890, Santa Clara, CA) equipped with flame ionization detector, integrator, and a 7683 autosampler. The oven temperature was programmed to hold at 240 °C for 4 min, increase to 288 °C at 3 °C/min and then increase to 298 °C at 2 °C/min. Helium was used as the carrier gas with a flow rate of 9.0 to 9.25 mL/min. Specific alkanes (nonacosane,C_{29}; hentriacontane, C_{31}; dotriacontane, C_{32}; hentriacontane, C_{33}; and $C_{36,}$ hexatriacontane) were identified by their retention times relative to known standards. Alkanes were quantified by peak areas compared to reference of C_{34} as an internal standard. Daily DMI was calculated using the following equation (Mayes et al., 1986b):

$$DMI = \left(\frac{Fi}{Fj} * RRj\right) \Big/ \left(Hi - \frac{Fi}{Fj} * Hj\right)$$

Where:

DMI = Daily herbage intake (kg DM/d)

RRj = Daily release rate of even-chained alkane (C_{32})

Fi and Hi = fecal and herbage concentration of odd-chained alkane (C_{33})

Fj and Hj = fecal and herbage concentration of even-chained alkane (C_{32})

The fecal recovery (99.1 %) was validated by Scaglia et al. (2005) on similar experimental conditions.

Statistical analysis

Seasonal data were analyzed as a randomized complete block design with forage as treatment. Due to the evaluation of the L treatment only in 2004, these data were only used in analysis of the 2004 data and not in across year analyses. Data were analyzed using repeated measures analysis of variance in PROC MIXED of SAS (SAS Institute Inc., 1996) with treatment as a fixed effect and year as the repeated variable for across year analysis and sampling date or period for seasonal trends. All means were reported as least squares means. The compound symmetry, cs, covariance structure provided the best fit data for analyses as compared to unstructured, un, and autoregressive, ar(1). Standard errors were calculated in SAS with the estimation of similar variances between treatments on any day. Differences were determined for the repeated measures using LSD adjusted *P*-values.

RESULTS AND DISCUSSION

Effect of sampling time on dry matter intake

There were no differences ($P = 0.59$) in DMI estimated between AM- and PM-collected samples and daily composite samples (Table 4-2). Diurnal variation of marker concentration in feces, especially in methods when markers are dosed once or twice daily, is a concern in DMI estimation (Burns et al., 1994). The development of the CRD was intended to decrease this variation along with other issues pertaining to daily dosing of markers including decreased labor and handling time of animals (Dove and Mayes, 1996). However, this method still raises concerns involving marker release rate and

recovery in feces (Burns et al., 1994). When using dosed, artificial alkanes, concerns with diurnal variation in recovery rate have been evoked due to the nature of natural, odd-chained alkanes to associate with the particulate phase and artificial, even-chained alkanes to associate with the liquid phase (Dove and Mayes, 1996). The findings of the current research are consistent with those of Lopez-Guerrero (2005). Lopez-Guerrero (2005) estimated the daily DMI of steers fed fescue hay using chromic oxide, and found that the DMI estimation with AM- or PM- fecal sampling (4.72 and 4.72 kg, respectively) was similar ($P = 0.99$) to the actual DMI (4.73 kg). Similarly, Hamaleers and Mayes (1998b) reported that the estimated daily DMI of dairy cattle consuming ryegrass (*Lolium perenne* L.) silage using the alkane ratio of two naturally occurring alkanes (C_{27}:C_{35}) was similar ($P = 0.34$) in AM (6.7 kg) and PM (7.0 kg) compared to group actual intake (6.8 kg). However, when using the ratio of C_{27} to the dosed alkane C_{36}, these authors reported that DMI estimation was higher ($P < 0.001$) with AM sampling (9.6 kg) compared to PM sampling (7.8 kg). These differences are due to diurnal variation in C_{36} excretion which is greater than the natural alkane, C_{27} (Hameleers and Mayes, 1998b). Also, Berry et al. (2000) found that DMI of forage (unspecified species) using C_{32} and C_{33} alkanes did not differ due to sampling time. Dry matter intake estimates for Brown Swiss cows fed in confinement did not differ when samples were collected at 0630 (15.4 ± 0.19 kg DM/d), 1330 (18.5 ± 2.0 kg DM/d), and 2030 (17.3 ± 1.2 kg DM/d). Lack of differences between sampling times was likely due to large variation and these authors suggest that 0630 was the optimal time for fecal sampling due to the smallest difference compared to actual DMI (15.3 ± 0.19 kg DM/d).

Forage sampling method

There was an effect of sampling method (WP vs. HP) in the estimated DMI of steers. Intake estimated from WP samples was lower compared to HP samples (Table 4-4). There was a trend ($P = 0.06$) in DMI (as a % BW) to be greater with HP compared to WP. It is well-documented that the quality of diet selected by the grazing animal will differ from clipped samples harvested at ground level (Weir, 1959; Coleman and Barth, 1973; De Vries, 1995). Dubbs et al. (2003) reported that forage samples collected from esophageally fistulated steers grazing tall fescue were lower in NDF and ADF and higher in CP compared to samples clipped by hand to ground level. The use of esophageally fistulated animal is not always practical in grazing research and thus collecting forage samples as close to that of what the animal selects is imperative to accurate assessments of the diet (Dubbs et al., 2003). De Vries (1995) reported that under range conditions hand-plucked samples, collected to closely mimic the selection of the grazing animals, were similar in nutritive value to samples collected from esophageal fistulated steers. When herbage allowance is not limiting, grazing animals select forage of greater nutritive value (Parsons et al., 1994). Therefore when estimation of DMI of grazing animals is to be estimated, it is apparent that sampling method affects DMI estimation.

Estimation of dry matter intake

In 2004 there was a treatment x period interaction ($P < 0.001$) for daily DMI. In May/June, DMI of steers grazing E- was higher than all treatments and Q and E+ were higher than L (Table 4-3). In July and September, DMI was higher in steers grazing E- than in those grazing Q, E+, or L, although absolute values of all fescues were lower compared to late spring. When reported as a % BW, DMI followed similar seasonal trends as total DMI (Table 4-3). During all three periods DMI of steers grazing E- was

higher than that of steers grazing Q, E+, and L. Dry matter intake of steers grazing E- and E+ followed similar seasonal trends decreasing from May to July and again in September, while DMI of steers grazing Q decreased from May to July and remained similar in September. Dry matter intake of steers grazing L was similar across periods. In May/June, DMI was highest for steers grazing E-, while DMI for steers grazing Q and E+ were higher than in those grazing L. In July and September DMI of steers grazing E- was higher than for Q, E+, and L.

Across treatments from May/June to July of 2004, herbage allowance (HA) decreased (Table 4-4). From May/June to July, NDF and ADF decreased and CP increased (Table 4-4). These changes in forage characteristics are likely due to stage of development. In May/June, steers were grazing paddocks were stems associated with reproductive structures were present. The presence of stems in this stage of development is associated with increase fiber and lower CP (Nelson and Moser, 1995). In early July, steers were grazing paddocks in which hay was previously harvested. This removed accumulated mature and stem material, resulting in forage of higher nutritive value, but less herbage mass. Average temperature humidity index (THI) indicated longer and more intense periods of mild stress in July (Table 4-5). Seman et al. (1997) reported that a negative correlation between solar radiation and grazing time of steers grazing both E+ and E- tall fescues. The increased stress during July may explain the decrease in DMI of steers grazing all fescue types despite the improved forage nutritive value. From July to September, DMI of steers grazing all fescue types decreased even though HA increased and nutritive value improved (Table 4-4). In addition, THI indicated lower heat stress conditions (Table 4-5). The decrease in DMI during this period, even though

environmental conditions and forage nutritive value improved, was unexpected. Interestingly, DMI was higher for steers grazing any of the fescue types when CP was the lowest and NDF and ADF were the highest. This is not consistent with previous research. Puoli et al. (1991) reported that the addition of 75 kg N/ha increased DMI of switchgrass (*Panicum virgatum* L.) and big bluestem (*Andropogon gerardii* Vitm.) hays by steers (11.4 and 16.1 %, respectively). Vazquez and Smith (2000) evaluated forage variables from 27 previously published studies and found that DMI was negatively correlated (r = -0.31) with diet NDF concentration.

In 2005 there was a treatment x period interaction for average DMI (Table 4-3). In June, average DMI of steers grazing E- and Q was higher than that of steers grazing E+. In August there was no difference in DMI of steers grazing E-, Q, and E+. In September, DMI of steers grazing E- was higher compared to those grazing Q and E+. When reported per unit of body weight, DMI followed similar trends to total DMI during the first two periods (Table 4-3). In early fall, DMI of steers grazing E- was higher compared to E+ with Q intermediate.

In 2005, HA and nutritive value (NDF, ADF, and CP) did not differ among treatments (Table 4-4), but there was a difference due to period ($P < 0.05$). Temperature Humidity Index indicated that steers were exposed to longer bouts of mild and moderate heat stress in August (Table 4-5). This is a possible explanation for the decrease in DMI. In September, DMI increased while HA was the lowest of the season. Nutritive value also improved in September suggesting that DMI followed trends of nutritive value and not HA. Herbage availability is a major factor influencing DMI of grazing animals (Vallentine, 2001). Stewart et al. (2006) found forage did not become limiting until HA

fell below 2.0 kg DM/kg BW on bahiagrass pastures. Redmon et al. (1995) observed a breaking point in DMI of steers grazing winter wheat (*Triticum aestivum* cv. 'Chisholm') in Oklahoma when HA fell below 2.0 to 2.4 kg DM/kg BW. Conversely, Dougherty et al. (1992) evaluated short term (1 hr periods) DMI of mature cows grazing E- tall fescue at different HA. When HA decreased from 4.2 to 1.3 kg/ha DMI did not decrease suggesting that HA was not affecting DMI (Dougherty et al., 1992). However, it should be noted that these DMI measurements were conducted over short time periods and may not reflect total DMI. Therefore, it appears that forage was not limiting DMI during any of the periods studied due to HA being higher than 2.0 kg/kg BW.

Dry matter intake estimates did not change through the season and remained at or below 15.1 g/kg BW through all periods for steers grazing L even though ADG of these steers (0.58 kg) were higher ($P < 0.05$) than E-, Q and E+ (0.54, 0.42, and 0.22 kg, respectively). Boland (2005) evaluated the botanical composition of L pastures and found encroachment of other grass species such as orchardgrass (*Dactylis glomerata* L.) and Kentucky bluegrass (*Poa pratensis* L.). Alkane profiles vary among forage species which can be beneficial in determining diet composition. However, if these profiles are not considered, DMI of other forages in addition to that of interest, can cause errors in the estimation of DMI (Dove and Mayes, 1996). Therefore DMI of steers grazing L may have been underestimated due to contamination of other species.

Previous research has reported a range of DMI of steers grazing tall fescue across different grazing conditions, some of which are similar to those in the current research. Scaglia et al. (2005) reported that daily DMI of steers (330±11 kg) grazing tall fescue using a ratio of dosed C_{32} to forage C_{33} alkanes in May and June of 2003. Dry matter

intake was 10.8 kg/d, and when adjusted to BW basis (32.7 g/kg BW) was higher than any treatment in the current research. Compared to the current research, CP and HA of the forage were higher (17.5 % and 9.32 kg/kg BW, respectively) and NDF lower (55 %). Lopez-Guerrero (2005) reported that the DMI of steers grazing tall fescue in August of 2003 using chromic oxide was 13.2 kg/d and when corrected for BW, 40.2 g/kg BW, which was also higher than values in the current research. Lopez-Guerrero (2005) reported similar CP concentration (14.6 %), but NDF was lower (45.0 %) and HA was higher (12.48 g DM/kg BW). Higher DMI reported by Scaglia et al. (2005) and Lopez-Guerrero (2005) may be explained by high HA on similar or higher nutritive value forage. At higher HA, animals can select for green leafy material and against stems (Heitschmidt and Stuth, 1991). The DMI of steers grazing E+ in the current research during early spring of 2004 and June and September of 2005 is consistent with that reported by Forcherio et al. (1995). Forcherio et al. (1995) reported that mature cows (459 ± 26 kg) with calves (average BW 98 ± 5 kg; 74 ± 5 d of age) grazing E+ tall fescue consumed 21.1 g DM/kg BW during the month of June on forage of similar CP, NDF, and ADF (9.8, 68.0 and 35.8 %, respectively) to the present study. Hannah et al. (1989) reported that DMI of steers (estimated using chromic oxide as an external marker) in the month of June grazing 'Kenhy', a low-endophyte tall fescue variety was 20.7 g/kg BW. No report of forage nutritive value was included, but these findings are lower than the DMI estimates in the current research during similar periods of the grazing season (Hannah et al., 1989).

Much of the reduced performance of animals grazing E+ tall fescue has related to reduced forage DMI. However, research results are conflicting. In the current research,

this suggestion is supported by the differences in DMI and ADG of steers grazing E- and E+ observed in 2004. However, in 2005 there were no differences across the year in DMI while ADG were different. Peters et al. (2002) reported that DMI of cows grazing E+ (26 g/kg BW) was similar ($P > 0.10$) to that of cows grazing E- tall fescue and orchardgrass (27 and 25 g/kg BW, respectively) during June when temperatures were not higher than 32 ºC. However, in August, when temperatures were above 32 ºC, DMI of cows grazing E+ (16 g/kg BW) was lower ($P < 0.05$) compared to E- and orchardgrass (19 and 20 g/kg BW, respectively). Even though DMI was similar in the early part of the grazing season, cows grazing E+ lost weight while cows that grazed E- gained weight. An interaction of environmental conditions and fescue toxicosis was suggested by Peters et al. (1992), where effects of toxicosis are increased as temperature increases. Similarly, Forcherio et al. (1992) reported that forage DMI of lactating multiparous cows grazing E+ from late May to July was 31% lower than DMI of cows grazing E-. In a second trial Forcherio et al. (1993), reported that lactating cows grazing E+ and E- tall fescues had similar DMI (22 g/kg BW) earlier in the spring (April to May). This is consistent with the present results for 2005 when DMI was similar among treatments in August and September when temperatures did not reach the level (32 °C) indicated by Paterson et al. (2002) as affecting DMI. However, during periods of both years when DMI of steers grazing E- and E+ differed, temperatures did not reach this level either. This would suggest another cause of decreased DMI, or that this threshold determined by Paterson et al. (2002) may not be useful in all environments. Temperature humidity index is a measure of heat stress that accounts for relative humidity. This index indicates times of mild stress during all periods of DMI estimation, and may partly explain the decrease

DMI during these periods. However, DMI was not different among treatments in August 2005 when THI indicated the longest period of mild or medium stress.

Hannah et al. (1990) reported that increasing ergovaline concentration in the diet of sheep by the inclusion of E+ tall fescue seed decreased ($P < 0.10$) ruminal and total tract OM, NDF, and cellulose digestibility. The lower digestibility of these fractions may have led to decreased DMI due to reduced passage rate from the rumen. Goetsch et al. (1987) reported that passage rate of particulates from the rumen decreased as proportion of E+ tall fescue hay in the diet increased. This may explain the differences in DMI of steers grazing E- and E+. However, in September of 2005, DMI was similar between steers grazing E- and E+ and increased from August to September despite the increasing levels of alkaloids in the forage (Figure 5-11). Seman et al. (1997) reported that time spent lying down and grazing of steers grazing E+ was lower ($P < 0.05$) compared to E- tall fescues. Similarly, Boland (2005) reported that steers grazing E+ spent more ($P < 0.05$) time standing and idling compared to steers on E- and Q. This increased idling time may partly explain the difference in DMI of E- and E+.

The use of non-ergot alkaloid-producing endophyte-infected fescue is a relatively new concept (Bouton et al., 2002), therefore little data are available where DMI of animals grazing these forages was estimated. The findings of the current research are inconsistent with those of Parish et al. (2003b). In spring, DMI of steers grazing E- (14.6 g/kg BW) and nonergot alkaloid-producing endophyte-infected 'Jesup' tall fescue (14.1 g/kg BW) was greater ($P < 0.10$) than that of steers grazing E+ (10.2 g/kg BW) tall fescue. In fall, DMI of steers grazing E- (14.0 g DM/kg BW) was higher ($P < 0.10$) than E+ (9.3 g DM/kg BW) while nonergot alkaloid-producing endophyte-infected 'Jesup' tall

fescue (11.2 g DM/kg BW) was intermediate (Parish et al., 2003b). In addition, the steers grazing E+ spent more time standing, idling, and consumed more water compared to steers grazing E- and the nonergot alkaloid-producing endophyte-infected tall fescue (Parish et al., 2003). Higher temperatures and humidity may have caused these changes in grazing behavior due to the location of this research, but weather data was not reported (Parish et al., 2003). The difference between DMI of steers grazing E- and Q in the current experiment was unexpected. This difference in DMI may be attributed to the indigestible fraction of Q (4.0 %) being higher compared to E- and E+ (2.2 and 2.5 %, respectively; Scaglia, personal communication). The higher indigestible fraction of Q likely caused a decrease in passage rate and, due to gut fill, may have limited DMI. Another explanation may involve differences in fescue variety. The fescue/endophyte combination used by Parish et al. (2003b) involves the same endophyte strain (AR542) as the current research, but infected in different fescue varieties. The E- and E+ fescue cultivar for this study was 'Kentucky 31' while Q is Q4508. The difference in variety may involve differences in palatability of forage of Q compared to E-.

CONCLUSIONS

Dry matter intake estimates of steers grazing E-, Q, E+, and L did not vary diurnally when using the dosed alkane C_{32} and natural alkane C_{33}. Therefore time of sampling does not appear to be a concern when estimating DMI. Additionally, due to differences in clipped and hand plucked sampling, it is important to closely represent the diet of the grazing animal in order to correctly estimate DMI. Steers grazing non-ergot alkaloid-producing endophyte-infected fescue did not exhibit signs of fescue toxicosis and had gains similar to those on E- tall fescues in previous research. However due to lower DMI compared to E- in the current research, caution should be taken when

selecting fescue varieties into which this endophyte is incorporate. Q5408 in particular, does not appear to be an optimal fescue variety.

TABLES

Table 4-1. Dry matter intake estimation periods and dates

Year	Period	Dosing/Sampling	Dates
2004	1	Dose	May 21
		Forage	May 26 – June 1
		Fecal	May 28 –June 3
	2	Dose	July 2
		Forage	July 7 – 13
		Fecal	July 9 – 15
	3	Dose	August 27
		Forage	September 1 – 7
		Fecal	September 2 – 9
2005	1	Dose	June 8
		Forage	June 13 – 19
		Fecal	June 15 – 21
	2	Dose	July 27
		Forage	August 1– 7
		Fecal	August 3– 9
	3	Dose	September 16
		Forage	September 20 – 26
		Fecal	September 22 – 28

Table 4-2. Least squares means for DMI estimates as affected by forage sampling method and fecal sampling method.

	Treatment[1]						
Item	E-	Q	E+	L	SE	AVG	SE
Forage Selection Method [2,3]							
DMI, kg							
Whole Plant	5.58[d]	4.92[d]	4.60[b]		0.15	5.04[b]	0.09
Hand Plucked	6.61 [c]	5.54[c]	5.40[a]		0.15	5.85[a]	0.09
DMI, g/kg BW							
Whole Plant	20.6	16.9	15.9		0.04	17.8[d]	0.02
Hand Plucked	22.3	17.7	17.5		0.04	19.1[c]	0.02
Diurnal Sampling[4]							
DMI, kg							
AM	9.41	5.91	6.52	4.67	0.19	6.63	0.09
PM	9.48	6.16	6.54	4.60	0.19	6.69	0.10
Composite	9.55	5.96	6.44	4.65	0.19	6.66	0.09
DMI, g/kg BW							
AM	32.6	19.8	22.9	15.0	0.10	22.6	0.05
PM	32.8	20.5	22.9	14.8	0.10	22.8	0.05
Composite	33.0	19.9	22.6	15.1	0.10	22.6	0.05

[1] Treatments: E+ = Kentucky 31 endophyte infected tall fescue; E- = endophye free tall fescue; Q = Q4508-AR542 non-ergot alkaloid producing- endophyte-infected tall fescues; L = Lakota prairie grass.
[2] May 26 to June 1, 2004.
[3]Forage Selection Method: whole plant = plant clipped approximately 2 cm above soil surface; hand plucked = plucking approximate 8 cm of the top of canopy.
[4] Diurnal Sampling: May 26 to June 1, 2004; AM = 0800; PM = 1700; Composite = A.M + PM composite sample, using hand plucked.
[ab] Within a column, means without a common superscript letter differ, $P < 0.05$.
[cd] Within a column, means without a common superscript letter differ $P < 0.10$.

Table 4-3 Average DMI of steers grazing Kentucky 31 endophyte infected and endophye free, and Q4508-AR542 non-ergot alkaloid-producing endophyte-infected tall fescues, and Lakota prairie grass

	2004		2005	
Period[1]/ Treatment[2]	DMI[3], kg	DMI[3], g/kg BW	DMI[3], kg	DMI[3], g/kg BW
Period 1				
E-	7.94[a]	27.6[a]	6.74[b]	24.6[a]
Q	5.96[bc]	19.9[cd]	6.72[b]	23.4[ab]
E+	6.44[bc]	22.6[bc]	4.88[d]	19.0[d]
L	4.70[e]	15.1[fg]		
Period 2				
E-	6.48[b]	23.2[b]	5.73[c]	20.6[cd]
Q	4.33[e]	14.3[fg]	5.45[c]	19.1[d]
E+	4.66[e]	15.9[ef]	5.51[c]	20.8[cd]
L	4.56[e]	14.2[fg]		
Period 3				
E-	5.67[c]	18.1[de]	7.54[a]	24.9[a]
Q	4.26[e]	12.9[g]	6.79[b]	21.8[b]
E+	4.13[e]	13.3[g]	6.68[b]	23.6[ab]
L	4.72[e]	13.2[g]		
SE	0.37	1.01	0.17	0. 77

[1] Period: 2004, 1 = May 26 to June 1; 2 = July 7 to 13; 3 = September 1 to 7; 2005, 1 = June 13 to 19; 2 = Aug 1 to 7; 3 = September 20 to 26.
[2] Treatments: E+ = Kentucky 31 endophyte infected tall fescue; E- = endophye free tall fescue; Q = Q4508-AR542 non-ergot alkaloid producing- endophyte-infected tall fescues; L = Lakota prairie grass.
[3]Treatment X period interaction, $P < 0.001$.
[abcdefg] Within a column, means without a common superscript letter differ, $P < 0.05$.

Table 4-4 Herbage allowance (HA) and nutritive value of Kentucky 31 endophyte infected and endophye free, and Q4508-AR542 non-ergot alkaloid-producing endophyte-infected tall fescues, and Lakota prairie grass

	2004				2005			
Period[1]/Treatment[2]	HA[3], kg/kg BW	CP[4], % of DM	NDF[3], % of DM	ADF[3], % of DM	HA[5], kg/kg BW	CP[3], % of DM	NDF[3], % of DM	ADF[3], % of DM
Period 1								
E-	7.9	8.8[f]	69.1	39.8	3.70	11.4	67.1	33.3
Q	7.2	8.7[f]	68.9	38.7	4.23	11.1	66.7	33.6
E+	6.1	8.2[f]	71.2	41.7	6.40	10.4	64.1	34.1
L	5.1	8.7[f]	71.9	40.3				
AVG	6.6[a]		70.3[a]	40.2[a]	4.78[a]	11.0[a]	66.0[a]	33.9[a]
Period 2								
E-	2.8	12.1[g]	59.9	31.4	5.70	11.0	66.8	35.1
Q	2.3	13.4[g]	60.4	31.9	4.88	12.3	65.6	33.6
E+	3.6	12.4[g]	60.7	32.7	5.40	10.8	64.9	36.5
L	2.8	16.7[h]	58.2	32.9				
AVG	2.9[c]		59.8[b]	32.2[b]	5.33[a]	11.3[a]	65.8[a]	35.1[a]
Period 3								
E-	4.2[e]	17.6[h]	59.4	28.3	2.33	15.4	54.5	26.9
Q	3.4[e]	17.8[h]	60.1	28.5	2.76	15.9	56.3	28.2
E+	7.1[d]	17.7[h]	59.8	28.9	2.90	14.3	52.6	29.0
L	5.0[de]	18.1[h]	59.2	31.7				
AVG	4.9[b]		59.6[b]	29.3[c]	2.66[b]	15.2[b]	54.5[b]	28.0[b]
SE	1.1	0.91	0.63	0.60	1.03	0.50	1.5	0.50

[1] Period: 2004, 1 = May 26 to June 1; 2 = July 7 to 13; 3 = September 1 to 7; 2005, 1 = June 13 to 19; 2 = Aug 1 to 7; 3 = September 20 to 26.
[2] Treatments: E+ = Kentucky 31 endophyte infected tall fescue; E- = endophye free tall fescue; Q = Q4508-AR542 non-ergot alkaloid producing- endophyte-infected tall fescues; L = Lakota prairie grass.
[3] Period effect, $P < 0.001$.
[4] Treatment x period interaction, $P < 0.10$
[5] Period effect, $P < 0.10$.
[abc] Within a column and period, means without a common superscript letter differ, $P < 0.05$.
[de] Within a column and period, means without a common superscript letter differ, $P < 0.10$.
[fgh] Within a column, means without a common superscript letter differ, $P < 0.05$.

Table 4-5. Temperature Heat Index (THI) during the periods of DMI estimation.

	THI[1]					
	2004			2005		
Time[2]	Period 1[3]	Period 2	Period 3	Period 1	Period 2	Period 3
0:00	17.62	18.68	16.61	16.7	20.2	15.8
1:00	17.17	18.24	16.15	15.6	19.6	15.7
2:00	17.36	17.55	15.81	15.1	19.1	15.6
3:00	17.14	17.33	15.77	14.7	18.6	15.7
4:00	17.07	17.05	15.75	14.1	18.2	15.7
5:00	17.23	17.00	15.85	13.7	17.9	15.2
6:00	16.58	16.93	15.89	13.9	17.7	15.2
7:00	16.00	16.97	15.73	15.9	17.6	15.4
8:00	17.03	17.96	16.06	18.2	17.7	16.3
9:00	18.41	20.15	17.18	20.1	18.2	17.9
10:00	19.34	22.51	18.62	21.5	19.4	20.5
11:00	19.90	23.75	20.63	22.2	21.1	22.6
12:00	20.88	24.73	21.62	22.3	23.3	23.4
13:00	21.66	25.14	22.03	22.3	25.1	23.9
14:00	22.34	25.34	22.26	22.9	26.0	24.1
15:00	22.19	25.50	22.31	23.2	26.4	23.9
16:00	22.23	25.52	22.28	23.3	26.5	23.8
17:00	22.39	24.89	22.30	23.2	25.7	23.4
18:00	22.49	25.04	22.19	22.7	25.5	22.9
19:00	21.74	24.91	21.81	22.1	25.6	21.5
20:00	20.87	24.29	20.61	20.6	25.7	19.9
21:00	19.35	22.58	18.46	18.5	25.3	18.6
22:00	17.69	20.21	17.66	17.9	23.0	17.8
23:00	17.29	19.12	17.38	17.0	21.4	17.1

[1] THI = Air Temp ºF - (0.55 – (0.55 * RH % / 100)) * (Air Temp ºF – 58.8)
Mild stress = 20.0 to 26.3; Medium stress = 26.3 to 38.0; Severe stress <38.0

[2] Time: Average of hour across each day of intake period.

[3] In 2004, Period 1 = May 26 to June 1; Period 2 = July 7 to 13; Period 3 = September 1 to 7; In 2005, Period 1 = June 13 to 19; Period 2 = Aug 1 to 7; Period 3 = September 20 to 26.

LITERATURE CITED

Berry, N. R., M. R. L. Scheeder, F. Sutter, T. F. Krober, and M. Kreuzer. 2000. The accuracy of intake estimation based on the use of alkane controlled-release capsules and faeces grab sampling in cows. Ann. Zootech 49: 3-13.

Boland, H. T. 2005. Grazing behavior of beef steers grazing endophyte-infected, endophyte-free, and novel endophyte infected tall fescue, and lakota prairie grass. M.S. Thesis., Virginia Tech, Blacksburg, VA.

Bouton, J. H., G. C. M. Latch, N. S. Hill, C. S. Hoveland, M. A. McCann, R. H. Watson, J. A. Parish, L. L. Hawkins, and F. N. Thompson. 2002. Reinfection of tall fescue cultivars with non-ergot alkaloid-producing endophytes. Agron. J. 94: 567-574.

Burns, J. C., K. R. Pond, and D. S. Fisher. 1994. Measurement of forage intake. In: J. G.C. Fahey, M. Collins, D. R. Mertens and L. E. Moser (eds.) Forage Quality, Evaluation, and Utilization. p 494-532. Am. Soc. of Agro.n Inc.; Crop Sci. Soc. Am. Inc.; Soil Sci. Soc. Am. Inc., Madison, WI, USA.

Coleman, S. W., and K. M. Barth. 1973. Quality of diets selected by grazing animals and its relation to quality of available forage and species composition of pastures. J. Anim. Sci. 36: 754-761.

De Vries, M. F. W. 1995. Estimating forage intake and quality in grazing cattle: A reconsideration of the hand-plucking method. J. Range Manag. 48: 370-375.

Dougherty, C. T., N. W. Bradley, L. M. Lauriault, J. E. Arias, and P. L. Cornelius. 1992. Allowance-intake relations of cattle grazing vegetative tall fescue. Grass and Forage Sci. 47: 211-219.

Dove, H., and R. W. Mayes. 1996. Plant wax components: A new approach to estimating intake and diet composition in herbivores. J. Nutr. 126: 13-26.

Dubbs, T. M., E. S. Vanzant, S. E. Kitts, R. F. Bapst, B. G. Fieser, and C. M. Howlett. 2003. Characterization of season and sampling method effects on measurement of forage quality in fescue-based pastures. J. Anim Sci. 81: 1308-1315.

Elizalde, J. C., J. D. Cremin, Jr., D. B. Faulkner, and N. R. Merchen. 1998. Performance and digestion by steers grazing tall fescue and supplemented with energy and protein. J. Anim. Sci. 76: 1691-1701.

Forcherio, J. C., G. E. Catlett, J. A. Paterson, M. S. Kerley, and M. R. Ellersieck. 1995. Supplemental protein and energy for beef cows consuming endophyte-infected tall fescue. J. Anim. Sci. 73: 3427-3436.

Forcherio, J. C., J. A. Paterson, and M. S. Kerley. 1992. Effect of source of supplemental energy and level of rumen undegradable protein on forage intake and performance of cow-calf pairs grazing endophyte-infected tall fescue pasture. J. Anim. Sci. 70(Suppl): 182(Abstr.).

Forcherio, J. C., J. A. Paterson, and M. S. Kerley. 1993. Effects of supplemental energy or protein on rumen parameters and performance of first and second partum beef cows grazing tall fescue. J. Anim. Sci. 71(Suppl. 1): 90 (Abstr.).

Goetsch, A. L., A. L. Jones, S. R. Stokes, K. W. Beers, and E. L. Piper. 1987. Intake, digestion, passage rate and serum prolactin in growing dairy steers fed endophyte-infected fescue with noninfected fescue, clover or wheat straw. J. Anim. Sci. 64: 1759-1768.

Hameleers, A., and R. W. Mayes. 1998. The use of n-alkanes to estimate supplementary grass silage intake in grazing dairy cows. J. Agric. Sci. 131: 205-209.

Hannah, S. M., J. A. Paterson, J. E. Williams, M. S. Kerley, and J. L. Miner. 1990. Effects of increasing dietary levels of endophyte-infected tall fescue seed on diet digestibility and ruminal kinetics in sheep. J. Anim. Sci. 68: 1693-1701.

Hannah, S. M., M. T. Rhodes, J. A. Paterson, M. Kerley, J. E. Williams, and K. E. Turner. 1989. Influence of energy supplementation on forage intake, digestibility and grazing time by cattle grazing tall fescue. Nutr. Rep. Int. 40: 1153-1157.

Heitschmidt, R. K., and J. W. Stuth. 1991. Grazing Management: An Ecological Perspective. Timber Press, Portland, OR.

Judkins, M. B., D. W. Holcombe, B. W. Hess, L. J. Krysl, M. A. Branine, and J. D. Hess. 1997. Ardacin for steers grazing endophyte-free fescue pasture: effects on live weight gain, forage intake, nitrogen and fiber digestion, ruminal fluid kinetics, ruminal fermentation, and serum hormones and metabolites. J. Anim. Sci. 75: 1100-1111.

Lippke, H. 2002. Estimation of Forage Intake by Ruminants on Pasture. Crop Sci 42: 869-872.

Lopez-Guerrero, I. 2005. Estimating Forage Mass of Tall Fescue Pastures and Dry Matter Intake and Digestibility of Fescue Forages by Beef Steers, Ph.D. Dissertation, Virginia Tech, Blacksburg, VA.

Mayes, R. W., and C. S. Lamb. 1984. The possible use of n-alkanes in herbage as indigestible fecal markers. Proc. Nutr. Soc. 43: 39A (abstract).

Mayes, R. W., C. S. Lamb, and P. M. Colgrove. 1986. The use of dosed and herbage n-alkanes as markers for the determination of herbage intake. J. Agric. Sci. 107: 161-170.

McCracken, B. A., L. J. Krysl, K. K. Park, D. W. Holcombe, and M. B. Judkins. 1993. Steers grazing endophyte-free tall fescue: Seasonal changes in nutrient quality, forage intake, digesta kinetics, ruminal fermentation, and serum hormones and metabolites. J. Anim. Sci. 71: 1588-1595.

Nelson, C. J., and L. E. Moser. 1995. Plant factors affecting forage quality. In: G. C. Fahey, Jr., M. Collins, D. R. Mertens and L. E. Moser (eds.) Forage Quality, Evaluation, and Utilization. p 115-154. Am. Soc. of Agron. Inc; Crop Sci. Soc. Am. Inc.; Soil Sci. Soc. Am. Inc., Madison, WI.

Owens, F. N., and C. F. Hanson. 1992. External and internal markers for appraising site and extent of digestion in ruminants. J. Dairy Sci. 75: 2605-2617.

Parish, J. A., M. A. McCann, R. H. Watson, N. N. Paiva, C. S. Hoveland, A. H. Parks, B. L. Upchurch, N. S. Hill, and J. H. Bouton. 2003. Use of nonergot alkaloid-producing endophytes for alleviating tall fescue toxicosis in stocker cattle. J. Anim Sci. 81: 2856-2868.

Parsons, A. J., J. A. Newman, P. D. Penning, A. Harvey, and R. J. Orr. 1994. Diet preference of sheep: effects of recent diet, physiological state and species abundance. J. Anim. Ecol. 63: 465-478.

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

Peters, C. W., K. N. Grigsby, C. G. Aldrich, J. A. Paterson, R. J. Lipsey, M. S. Kerley, and G. B. Garner. 1992. Performance, forage utilization, and ergovaline consumption by beef cows grazing endophyte fungus-infected tall fescue, endophyte fungus-free tall fescue, or orchardgrass pastures. J. Anim. Sci. 70: 1550-1561.

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

Puoli, J. R., G. A. Jung, and R. L. Reid. 1991. Effects of nitrogen and sulfur on digestion and nutritive quality of warm-season grass hays for cattle and sheep. J. Anim. Sci. 69: 843-852.

Redmond, L. M., D. L. Cross, J. R. Strickland, and S. W. Kennedy. 1994. Efficacy of domperidone and sulpiride as treatment for fescue toxicosis in horses. Am. J. Vet. Res. 55: 722-729.

SAS. 1996. SAS statistics user's guide, Version 6, Cary, N.C.

Scaglia, G., H. T. Boland, I. Lopez-Guerrero, R. K. Shanklin, and J. P. Fontenot. 2005. Use of alkanes to estimate dry matter intake of beef steers grazing high quality pastures. Proc. XX International. Grassland Congress: 250.

Seman, D. H., J. A. Stuedemann, and J. E. Anderson. 1997. Spectral analysis of bovine grazing behavior on *Neotyphodium coenophialum* infested tall fescue. Appl. Anim. Behav. Sci. 54: 73-87.

Sollenberger, L. E., and D. J. R. Cherney. 1995. Evaluating forage production and quality. In: R. F. Barnes, D. A. Miller and C. J. Nelson (eds.) Forages. Vol II: The Science of Grassland Agriculture. p 97-110. Iowa State University Press, Ames, IA, USA.

Stewart, R. L., Jr. , L. E. Sollenberger, J. C. B. Dubeux, Jr., J. M. B. Vendramini, S. M. Interrante, and Y. C. Newman. 2006. Herbage and animal responses to management intensity of continuously stocked bahiagrass pastures. Agron. J. In Press.

Vallentine, J. F. 2001. Grazing Management. 2nd ed. Academic Press, New York.

Vazquez, O. P., and T. R. Smith. 2000. Factors Affecting Pasture Intake and Total Dry Matter Intake in Grazing Dairy Cows. J. Dairy Sci. 83: 2301-2309.

Weir, W. C. 1959. Selective grazing by sheep as shown by a comparison of the chemical composition of range and pasture forage obtained by hand clipping and that collected by esophageal-fistulated sheep. J. Anim. Sci. 18: 641-649.

CHAPTER 5
COPPER AND COPPER-ZINC SUPEROXIDE DISMUTASE STATUS IN ANIMALS GRAZING THREE FESCUE TYPES AND LAKOTA PRAIRIE GRASS

ABSTRACT

A grazing study was conducted to measure Cu status of steers grazing three tall fescue [*Schedonorus arundinaceus* (Schreb.) Dumort.] types ['Kentucky-31' endophyte-infected (E+), endophyte free (E-) tall fescues, and Q4508-AR542 non-ergot alkaloid-producing endophyte-infected tall fescue (Q)], and 'Lakota' prairie grass (L; *Bromus catharticus* Vahl.). Forage Cu was concentration was greater ($P < 0.05$) in L compared to all fescues and E- was greater ($P < 0.05$) than E+ in 2004. No differences in forage Cu were observed among treatments in 2005 or across seasons ($P > 0.10$). In 2004, Cu intake was highest ($P < 0.001$) for steers grazing E- and lowest for E+ in 2004, and it was similar across fescue types in 2005. Serum Cu was not different among treatments in 2004 ($P = 0.81$), but in 2005, serum Cu of steers grazing E- and Q was greater than E+ ($P < 0.05$). In 2004, liver Cu levels of cattle grazing E- were greater ($P < 0.05$) than for those grazing E+, while liver Cu levels of steers grazing Q and L were intermediate, but differences were not detected among treatments in 2005 ($P = 0.86$). Cu/Zn superoxide dismutase (SOD) enzymatic activity did not differ among treatments in 2004 or 2005 ($P = 0.79$ and 0.80, respectively). No differences in relative Cu/Zn superoxide dismutase mRNA expression were observed among treatments in either year. These results suggest that Cu intake of cattle grazing all forages were sufficient to maintain liver Cu status, but

not replenish deficient liver Cu. Also, endophyte status of forage did not affect Cu/Zn SOD enzymatic activity or relative mRNA expression of steers grazing E+.

INTRODUCTION

Tall fescue [*Schedonorus arundinaceus* (Schreb.) Dumort.] is a vital component of the beef cattle industry throughout the southeastern United States. Alkaloids produced by the infection of tall fescue with the fungal endophyte *Neotyphodium coenophialum* have been associated with several disorders in grazing animals, collectively referred to as fescue toxicosis (Hoveland, 2003). Dennis et al. (1998) reported a decrease in Cu levels in plant material from endophyte-infected tall fescue compared to endophyte-free tall fescue in both greenhouse and field experiments. Copper is an essential micromineral and is a component of several metalloproteins. Animals grazing E+ tall fescue have decreased immune response as well as lower ceruloplasmin and serum Cu compared to animals grazing E- tall fescue (Saker et al., 1998). Coffey et al. (2000) reported supplementation with CuO increased ceruloplasmin and serum Cu levels in animals grazing E+ tall fescue.

Reactive oxygen metabolites, such as superoxide ($O_2^{-}\cdot$), are unavoidable products of normal metabolic function. These compounds are not always harmful and are involved in the physiological function of several enzymes and used by phagocytes to kill bacteria (Halliwell, 1987). However, an imbalance of reactive oxygen metabolites production and disposal, can lead to the initiation of oxidative stress. Copper is a component of Cu/Zn superoxide dismutase (SOD), a vital antioxidant enzyme that scavenges one of the reactive oxygen species, $O_2^{-}\cdot$. Copper also functions in eukaryotic gene expression by activation and repression of gene transcription through regulatory metal-binding proteins that interact with metal-responsive elements (MRE) in MRE-

regulated genes, including Sod1 which is responsible for SOD (Uauy et al., 1998). Therefore, decreased Cu status of animals grazing E+ tall fescue could possibly increase oxidative stress leading to decreased animal performance.

The objective of this research was to determine the Cu levels in forage and in bovine liver and serum samples, as well as quantify Sod1 expression in hepatic tissue and SOD activity in red blood cells in beef cattle grazing three fescue types and 'Lakota' prairie grass (*Bromus catharticus* Vahl.).

METHODS AND MATERIALS

Experimental site

A 2-yr grazing trial was conducted during 2004 and 2005. This experiment was conducted at Virginia Tech's Kentland Farm located west of Blacksburg, VA (37°11' N, 80°35' W).

Treatments and design

Treatments were different forage types and included alkaloid-producing endophyte infected 'Kentucky 31' tall fescue infected with wild-type alkaloid producing endophyte (E+), endophyte-free Kentucky 31 tall fescue (E-), non-ergot alkaloid-producing endophyte Q4508-AR542 tall fescue (Q), and Lakota prairie grass (L; *Bromus catharticus* Vahl.). Prairie grass was not used during the 2005 season. Treatments were arranged in three replicates of a randomized complete block design with pasture as the experimental unit (n=12, 2004; n=9, 2005).

Pasture and animal management

Pastures were managed under rotational stocking during the grazing seasons of 2004 (135 d) and 2005 (136 d). Grazing began on May 5 of the 2004 season and May 3 of the 2005 season. Each pasture was subdivided into six paddocks (approximately 0.20

ha), and animal movement from paddock to paddock was determined by visual appraisal of available forage (based on residual height of 7 to 10 cm). Pastures were seeded between September 20 and 25, 2002 at seeding rates of 39 kg/ha for L and 25 kg/ha for all the fescues. Due to stand failure, the E- treatment was reseeded on March 30, 2003. All pastures were fertilized according to soil test recommendations. Each year prior to grazing 33.6 kg/ha of liquid N was applied to all pastures. In 2004, an additional 56 kg/ha of 46-0-0 fertilizer was applied to all pastures on August 20 and to L after each of the paddocks had been grazed by the steers for 7 to10 d. Hay was harvested from three of the six paddocks in each pasture on June 6 2004 and May 15 2005. The remaining paddocks were clipped after animals were removed in late spring to eliminate reproductive tillers.

For the 2004 grazing season, steers were purchased December 1, 2003, at a Virginia feeder cattle sale and shipped to Smithfield Farm, Virginia Tech, Blacksburg, VA. Steers were kept in dry lot from the date of purchase until May 4, 2004. From January 6 to May 4, steers were fed a diet consisting of 51% barley straw, 37% corn, and 5% molasses, with the remaining portion containing soybean meal, feather meal, and urea. Average daily gain for steers during the drylot period was 0.64 kg. In 2005, steers were purchased on April 19 at a Virginia feeder cattle sale and shipped to Kentland Farm, Virginia Tech, Blacksburg, VA. Thirty-six crossbred steers with initial BW of 272 ± 19 kg and 244 ± 17 kg (for 2004 and 2005, respectively) were blocked by weight and randomly allotted within block to the four treatments. Each pasture had three animals, two of which were randomly assigned as tester animals for sampling. Steers did not have access to Cu or Zn mineral supplementation to limit Cu intake to only that from the

forage. Steers did not have access to shade. Each year, steers were vaccinated for Infectious Bovine Rhinotracheitis (IBR), Bovine Viral Diarrhea (BVD), Bovine Respiratory Syncitial Virus (BRSV), Parainfluenza3 (PI3) with Pyramid 4® (Fort Dodge Animal Health, Fort Dodge, IA) and with Vision 7® (Intervet, Millsboro, DE) for *Clostridium chauvoei* (Blackleg), *C. septicum* (Malignant edema), *C. novyi* (Black disease), *C. sordellie* and *C. perfringens* Types C and D (Enterotoxemia). Steers were treated with Cydectin® (moxidectin; Fort Dodge Animal Health, Fort Dodge, IA) for internal and external parasites on d 0 and 56 of the grazing season and with Elector® (spinosad; Elanco™ Animal Health, Greenfield, IN) as needed for external parasites. On approximately d 56 of both years animal were treated with Co-Ral Plus® insecticide ear tags (Diazinon and Coumaphos; Bayer HealthCare, Shawnee Mission, KS).

Animal and plant samples

Two steers in each pasture were randomly assigned as testers for sampling. On d 0 and every 28 d thereafter steers were weighed and blood was collected by jugular veinipuncture into heparinized and non-additive Vacutainer™ blood tubes for Cu analysis and SOD activity. On d 0, 56, and 112, liver biopsies were conducted on tester steers. During the 2005 season, liver biopsies were not collected until d 14. Biopsies were collected using a stainless steel, reverse cutting biopsy probe (5 mm i.d., 7 mm o.d; Sontec Instruments, Centennial, CO). A surgical site between the 11th and 12th rib was prepared on each steer. A lidocaine block was administered at the site of the incision, a small incision was made with a #10 scalpel blade, and the biopsy needle was directed ventrally and cranially toward the left elbow. Braunamid suture (#3; Braun Surgical GmbH, Horgensen Labs, Loveland, CO) was used to close the skin after the procedure.

Each liver sample was collected, divided into two subsamples, frozen and stored in liquid N until transported to the laboratory. Samples were stored at -80 °C until analyzed.

On days the steers were weighed, hand-plucked forage samples were collected for Cu analysis from those paddocks that the steers were entering. Plant material was dried in a forced-air oven at 60 °C for 48 h and ground through a 1-mm screen in a stainless steel Wiley Mill (Thomas Wiley Laboratory Mill Model 4, Arthur H. Thomas Co., Philadelphia, PA).

Laboratory Analysis

Mineral analysis

Blood samples from non-additive blood tubes were centrifuged at 2000 x *g* for 20 min and serum was collected. Serum was diluted 2:1 with deionized water for Cu and Zn analysis. Liver samples were weighed on a wet basis and 0.5 g of dried forage samples were weighed and placed in 50 mL digestions tubes. Liver and forage samples were then digested with 2:1 (vol:vol) nitric:perchloric acid (Muchovej et al., 1986). All samples were analyzed for Cu and Zn on an atomic absorption spectrometer (Perkin Elmer AAnalyst 800, Norwalk, CT).

Enzymatic activity

Copper/Zinc SOD activity was quantitatively determined in whole blood samples by the inhibition of superoxide-dependent reduction of cytochrome *c* by spectrometry (Shimadzu UV 265, Shimadzu Scientific, Columbia, MD) as described by McCord and Fridovich (1969). Briefly, superoxide was generated in a standard solution of xanthine and cytochrome *c* by the xanthine oxidase-dependent oxidation of xanthine and subsequent reduction of O_2. The standard assay was carried out in a 5.0 mL solution containing 150 µl of 1mM xanthine, 300 µL of 0.1 mM cytochrome *c* and 2.5 mL SOD

buffer. The spectrometer was calibrated by adding sufficient xanthine oxidase (approximately 2.5-5.0 μL) to the above solution to establish a rate of reduction of cytochrome *c* of 0.025 absorbance/min at 550 nm.

Whole blood samples were allowed to thaw at 4 °C and diluted to a 3:100 solution in deionized water. One hundred μL of the whole blood was added to the standard solution in a 1.0 cm cuvette and the predetermined amount of xanthine oxidase was added to initiate the reaction. Absorbance was plotted over time (approximately 2 min) and inhibition was calculated as the percent inhibition per minute. Under these conditions, the amount of SOD required to inhibit the rate of reduction of cytochrome *c* by 50% (a rate of 0.0125 absorbance unit/min) is defined as one unit (U) of activity. Enzymatic activity of Cu/Zn SOD is reported as units of SOD/mg of soluble protein. Soluble protein was determined using the biuret reaction (Gornall et al., 1949) and a standard curve was prepared using bovine hemoglobin.

Real-Time polymerase chain reaction

Total RNA was isolated from each sample using the TRI-Reagent manufacturer's protocol (Molecular Research Center, Inc., Cincinnati, OH). Briefly, the liver samples were transferred directly from the freezer to 12 mL Falcon polypropylene tubes containing 1 mL of TRI-REAGENT. The samples were homogenized using a tissue homogenizer (Tekmar Tissumizer® Model #SDT-1810; Cincinnati, OH) for 30 sec homongenization/10 sec ice incubation cycles until no visible particles remained. The samples were incubated on ice for approximately 5 min and split between two 2-mL microcentrifuge tubes containing 300 μL of chloroform. The samples were centrifuged for 15 sec and incubated at room temperature for 10 min prior to centrifugation at 12,000 x g for 15 min at 4 °C (Micomax Therma IEC microcentrifuge; Thermo Electron

Corporation, Waltham, MA). The clear aqueous phase was then removed and transferred to a new 2 mL microcentrifuge tube containing 750 µL of isopropanol, vortexed for 5 sec, and held at room temperature for 5 min. The samples were then centrifuged at 12,000 x g for 8 min at 4 °C. The supernatant was discarded, and the RNA pellet was washed with 1 mL of 75% ethanol. The samples were centrifuged at 7,500 x g for 5 min at 4 °C, and after centrifugation, the supernatant was discarded, and the tubes were inverted on a KimWipe® and air dried for 5 to 10 min. After drying, the pellet was resuspended in 70 µL of diethyl pyrocarbonate (DEPC) treated water. Total RNA was quantified with a spectrophotometer (Model U-2000, Hitachi Instrument, Japan) at 260/280 nm with the 260:280 ratio for each sample being between 1.7 and 1.8. Isolated RNA samples were stored at -80 °C until further analysis. Integrity of RNA samples was tested by running 5 µg of total RNA on a 1% denaturing agarose gel.

Total RNA from each liver sample was used to generate cDNA through a reverse transcription reaction (IMProm – II Reverse Transcriptase, Promega) in a PTC-200 Peltier DNA Engine (MJ Research, Reno, NV). Primers (Table 5-1) were designed for each gene, Sod1 and a housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) using Primer Express software (NIH, Bethesda, MD), for optimal use with Applied Biosystems Real-Time PCR Systems (Foster City, CA). Real-Time PCR was performed (Applied Biosystems 7300, Foster City, CA) using the relative quantification method. Liver cDNA samples from all steers were run in duplicate on each plate. Each 25 µL PCR reaction contained 2 µL of cDNA 1:30 dilution, 12.5 µL SYBR green master mix (Applied Biosystems), 9.5 µL water and 0.5 µL of forward and reverse primer at a 5

μ*M* concentration. Polymerase chain reaction was performed under the following conditions:

1. 50 °C for 10 min
2. 95 °C for 1 min
3. 60 °C for 1 min
4. Steps 2 and 3 repeated for 40 cycles

With the threshold cycle (Ct) values determined by the system, the relative quantification of Sod1 mRNA compared with the internal control gene GAPDH could be calculated according to the $2^{-\Delta\Delta Ct}$ method described by Livak and Schmittgen (2001).

Statistical analysis

Seasonal data were analyzed as a randomized complete block design with forage as treatment. Due to the evaluation of the L treatment only in 2004, these data were only used in analysis of the 2004 data and not in across year analyses. Data were analyzed using repeated measures analysis of variance in PROC MIXED of SAS (SAS Institute Inc., 1996) with treatment as a fixed effect and year as the repeated variable for across year analysis and sampling date for seasonal trends. All means were reported as least squares means. The compound symmetry, cs, covariance structure provided the best fit data for analyses as compared to unstructured, un, and autoregressive, ar(1). Standard errors were calculated in SAS with the estimation of similar variances between treatments on any day. Differences were determined for the repeated measures using LSD adjusted *P*-values. Due to large variation in initial liver mineral values, d 0 (2004) or d 14 (2005)

concentrations of Cu and Zn were used as a covariate. Stepwise regression analyses were performed using Cu intake as the dependent variable and DMI and forage Cu as independent variables in PROC STEPWISE of SAS (SAS Institute Inc., 1996).

RESULTS AND DISCUSSION

Forage Cu and Zn

There was a year effect on forage Cu with Cu being greater ($P < 0.05$) in 2005 compared to 2004 (4.8 vs. 3.9 μg/g). There was no year x treatment interaction ($P = 0.44$) for forage Cu. Across years, there was no difference ($P = 0.20$) between treatments, but Q and E- were numerically greater than E+ (Table5-2). During the 2004 season Cu concentrations were greatest ($P < 0.001$) in L, intermediate in E- and Q, and least in E+ forage. In 2005, there was no differences ($P = 0.19$) among treatments.

These Cu concentrations are below the recommended amount for beef cattle of 10 μg/g DM (NRC, 2000), but are similar to values of E+ and E- tall fescue (4.5 and 4.8 μg/g) previously reported in Virginia (Dennis et al., 1998). Malinowski et al. (2004) reported Cu levels in plant material of E- and AR542-infected tall fescues in P-limiting and P-sufficient conditions. Under P-limiting conditions, root Cu values were lower for AR542 compared to E-, but under P sufficient conditions, there were no differences. These conclusions suggested that soil P is influential in E+ Cu status (Malinowski et al., 2004). Soil P levels ranged from 17 to 35 ppm in E+ and Q pastures, and these values are considered sufficient for pasture production (Donohue, 1992). These P-sufficient soils may explain the lack of difference in forage Cu concentrations between fescue types. Although no literature is available comparing Cu concentrations in L and tall fescues, LaCasha et al. (1999) reported that the level of Cu in Matua prairie grass (*Bromus willdenowii* Kunth. Cv. Grasslands Matua) which is a similar grass to L, was greater than

for other forages such as alfalfa (*Medicago sativa* L.) and coastal bermudagrass (*Cynodon dactylon* (L.) Pers.) with 8, 7, and 5 µg/g, respectively. In addition, N fertilization increases forage Cu levels (Dennis et al., 1998). This may partly explain the higher forage Cu concentration in L due to higher fertilization.

In 2004, Cu level in L was greater than both E- and E+ on June 28 with Q intermediate (Table 5-3). Lakota was greater than all fescues on July 23 and August 20. All forages followed similar seasonal trends decreasing from late spring to early summer and then increasing gradually to mid summer, with a larger increase in late summer. In 2005, there were no differences in treatments on any sampling date, but all forages followed similar seasonal trends (Table 5-3). Forage Cu increased steadily from late spring to mid summer and gradually increased to late summer. Dennis et al. (1998) reported Cu values decreased from pre bloom to full bloom, and were highest in forage regrowth. In the present study, hay was harvested in early June of 2004 and late May of 2005. Therefore, forage was in pre bloom on May 28 (2004) before forage Cu declined to its lowest level. On June 24 (2004) and May 31 (2005) plants were in full bloom when forage Cu was minimal in both years. On subsequent sampling dates, Cu began to increase when forage present was regrowth.

Forage Zn concentrations were greater ($P < 0.05$) in 2004 than 2005. Both E- and Q had greater ($P < 0.05$) Zn E+ (Table 5-2). Over the two years, Zn concentrations averaged 17.6 µg/g, which is below the requirement of 30 µg/g for growing cattle (NRC, 2000), but within normal ranges of forages (Corah and Dargatz, 1996).

Copper and zinc status in steers

Copper intake

Copper intake was based on extrapolation of forage Cu concentration and DMI estimates from Chapter 4. There was a year, but no year x treatment effect on Cu intake ($P < 0.001$ and $P = 0.17$, respectively). Lower Cu intake in 2004 compared to 2005 (0.08 vs 0.16 µg Cu/kg BW) can be attributed to both animal intake (15.3 vs 21.5 g DM/kg BW, respectively; $P < 0.01$) and forage Cu level being lower in 2004 compared to 2005.

In 2004, there was a treatment x period interaction on Cu intake. In late spring, Cu intake was highest for steers grazing E-, and E+ was greater than Q and L (Table 5-4). In early summer, Cu intake of steers grazing E- was the highest among treatments and Cu intake of steers grazing L was greater than Q, which was greater than E+. In late summer, Cu intake was highest on L and Cu intake of steers grazing E was greater than Q and E+.

In 2005, there was a treatment x period interaction on Cu intake (Table 5-4). In June, Cu intake of steers grazing E- was greater than E+ with Q intermediate. There were no differences in Cu intake among treatments during the final two intake periods. In August, Cu intake of steers grazing E- and Q were similar to June while Cu intake of steers grazing E+ increased. In September, Cu intake of steers grazing E- increased and although not statistically different, Cu intake of steers increased on Q and E+.

Copper intake is a function of both Cu content of forage and DMI of the animal. Stepwise regression analysis included DMI in the model first and it explained 88% ($r^2 = 0.88$) of the variation in Cu intake. Although Cu levels in forage increased through the season of 2004, these increases were counteracted by decreased DMI of the steers on the

fescues causing a decrease in Cu intake. On L, DMI did not decrease across the season and therefore Cu intake actually increased from late spring to late summer. Unlike 2004, smaller variations in DMI and increasing forage Cu through the season resulted in less variation in Cu intake. Dennis et al. (1998) suggested that Cu deficiencies in cattle grazing E+ tall fescue were a result of low Cu levels in forage and decreased DMI. This is supported in the current research, where there was a trend (P = 0.12) for Cu intake of steers grazing E- to be greater than E+ (13.8 vs. 10.4 µg Cu/kg BW). In addition, Cu intake of steers grazing E- was numerically greater than Q (11.8 µg Cu/kg BW) suggesting possible Cu deficiency problems arising when animals graze non-ergot alkaloid-producing endophyte infected tall fescues. Throughout both seasons, forage Cu levels were below the NRC daily requirement of 10 µg/kg of diet. If the daily requirement of Cu is based on animals consuming 25 g DM/kg BW of a given diet, this would represent 250 µg/kg BW of Cu consumed Therefore, under the grazing conditions of this experiment, steers were not able to consume the daily requirement of Cu.

Serum and liver minerals

There was no treatment x year interaction (P = 0.18) or year effect (P =0.56) on serum Cu. During the 2004 season there was no effect of treatment on serum Cu (P = 0.87), but there was an effect due to sampling date (P < 0.001). Serum Cu was adequate (Wikse et al., 1992) for all treatments at the beginning of the grazing season and remained unchanged through June 25, decreased to marginally deficient levels on July 23 and gradually increased to sufficient levels by the end of the grazing season (Table 5-6).

During the 2005 season there was a treatment effect on serum Cu (P < 0.01) with serum Cu of steers grazing both E- and Q being greater than those grazing E+. At the beginning of the grazing season there was a trend (P = 0.09) for serum Cu of steers

grazing Q and E- to be greater than those grazing E+. There were differences among treatments on June 28 due to serum Cu of steers grazing Q being greater than those grazing E+ with E- intermediate (Table 5-6). On August 23 and September 20, Cu serum of steers grazing both Q and E- were greater than those grazing E+. Over the season, all treatments followed similar trends and there was an effect due to sampling date ($P < 0.001$). At the initiation of the grazing season, steers were marginally deficient in serum Cu and increased to sufficient levels by May 31. Serum Cu decreased slightly in steers grazing E- and E+ while those grazing Q remained unchanged, and all decreased to deficient levels on July 26. Serum Cu increased in all steers throughout the remainder of the grazing season with steers grazing Q and E- reaching sufficient levels of serum Cu on August 23 with those grazing E+ remaining marginally deficient.

There was no effect of year ($P = 0.87$), treatment ($P = 0.40$), or year x treatment ($P = 0.93$) interaction on liver Zn and values indicated sufficient levels (Puschner et al., 2004) ranging from 33.4 to 35.4 µg/g.

There was no treatment x year interaction for liver Cu ($P = 0.19$); however, there was a year effect ($P < 0.01$). Liver Cu was greater in 2005 compared to 2004 (25.4 vs 15.3 µg/g) and can be attributed to background prior to the experiment. Initial Cu tended ($P = 0.14$) to be greater in 2004 compared to 2005 (22.6 vs 13.2 µg/g). In 2004, steers were backgrounded together for 155 d prior to the experiment and liver Cu ranged from 6.6 to 24.7 µg/g. In 2005, steers began the experiment directly from a Virginia feeder cattle sale with a larger range (3.4 to 67.3 µg/g) in liver Cu. Also, Cu intake in 2005 was greater compared to 2004 possibly contributing to greater liver Cu in 2005.

During 2004, liver Cu did not differ ($P = 0.88$) among treatments on d 56, but by d 112 there was a treatment effect ($P < 0.01$). Liver Cu of steers grazing E- was greater than those grazing L and E+, with Q intermediate but greater than E+ (Table 5-7). Dennis et al. (1998) suggested lower Cu status of steers grazing E+ compared to E- forages was due to a decrease in DMI. The greater liver Cu of steers grazing E- is reflected in Cu intake across the season being greater for E-. At this level of DMI it appears than Cu is being stored in the liver. However over the grazing season, this was not enough to increase liver Cu to sufficient levels. Interestingly, liver Cu of steers grazing Q was similar to those grazing E- by d 112 even though DMI of steers grazing Q was lower than those grazing E- throughout the season. In 2004, there was a trend ($P = 0.15$) for differences in sampling dates with average Cu being greater on d 112 compared to d 56. In 2005 there was no effect of treatment on liver Cu on d 56 ($P = 0.33$) or d 112 ($P = 0.86$). This is likely due to the similarities in Cu intake across the season on all treatments. There was an effect of sampling date in 2005 on liver Cu ($P < 0.05$) with Cu being greater on d 56 compared to d 112. This was unexpected due to Cu intake increasing across the season. Grings and Poland (2000) investigated the change in liver Cu of steers consuming alfalfa hay or wheatgrass (*Agropyron* spp.) hay (7 and 2 µg/g Cu, respectively) at an average DMI of 21 g/kg BW. Copper intake was calculated to be 148.8 and 42.5 µg/kg BW, on alfalfa and wheatgrass hays, respectively. After 91 d steers were harvested, liver samples taken and Cu determined. Liver Cu for steers consuming alfalfa and wheatgrass hays was 38.0 and 24.3 µg/kg DM, respectively. It is difficult to compare these values to the current experiment because initial liver Cu is unknown and liver Cu values are reported on a DM basis (Grings and Poland, 2000). However, it can

be noted that in the present study (2004), differences in Cu intake (Table 5-4) between steers grazing E- and E+ were not as large as those reported by Grings and Poland (2000), and similarly, differences in liver Cu were not as large (Table 5-7).

In stressed situations, plasma Cu concentration increases (Cousins, 1985), but these increases are not as pronounced in Cu-deficient cattle (Gengelbach et al., 1997). Despite the severe Cu deficiency indicated by low liver Cu in 2004, serum Cu levels remained sufficient for the first 56 d and dropped to slightly deficient throughout the remainder of the grazing season. Gengelbach et al. (1997) reported plasma Cu decreased from 0.72 to 0.24 μg/mL in steers consuming a Cu-deficient diet (4.5 μg/g Cu) over a 70-d period. Ward et al. (1993) reported serum Cu of steers consuming a diet of 6.2 μg/g Cu did not become deficient after 98 d on the diet. In the current experiment, forage Cu ranged from 3.7 to 4.9 μg/g in 2004 and 4.6 to 5.0 μg/g in 2005. Although forage Cu levels in 2004 were lower than those reported by Gengelbach et al. (1997) and Cu levels in 2005 were slightly greater, serum Cu responded similarly to those of cattle on a greater Cu diet (Ward et al., 1993) and remained relatively static throughout the grazing season.

Underwood and Shuttle (1999) suggest liver Cu serves as an indicator of the prior diet and the degree of storage depletion, but not an indicator of functional Cu status at critical sites, and only when liver Cu reaches levels where it cannot be mobilized does plasma Cu or transport fall below normal. At this point animals are deficient and if Cu continues to decrease, function of Cu-dependent process becomes compromised. Although there are brief periods of deficient serum Cu in both years, this suggests that Cu function should not have been greatly affected.

Cu/Zn SOD enzymatic activity

There was no year (P = 0.35) or treatment x year interaction (P = 0.78) on whole blood Cu/Zn superoxide dismutase (SOD) enzymatic activity. During 2004 there was no treatment (P = 0.79) effect, but there was an effect (P < 0.001) due to sampling date. At the initiation of grazing, SOD activity (2.6 U/mg soluble protein) decreased to d 56 (1.6 U/mg soluble protein) and remained similar on d 112 (Figure 5-1). In 2005, there was no effect of treatment (P = 0.80) on SOD activity, but activity was affected by sampling date (P < 0.01). Superoxide dismutase activity did not change from d 21 (2.5 U/mg soluble protein) to d 56, but decreased on d 112 (Figure 5-2). Ward and Spears (1997) reported that erythrocyte SOD activity was similar in bull calves after consuming a Cu-deficient diet (6.9 μg/g) compared to those consuming a Cu sufficient diet (14.4 μg/g) for a 168-d period. Suttle and McMurray (1983) collected data from experiments where ruminants (steers, lambs, and ewes) had either been depleted of Cu or repleted with Cu at various rates and erythrocyte SOD had been monitored. From these data, these authors reported that erythrocyte SOD activity declined at only 33 to 14 % the rate of plasma Cu. Paynter (1987) suggested that erythrocyte SOD is affected more slowly than plasma Cu, and therefore prolonged Cu deficiency is better indicated by SOD activity. In 2004 liver Cu indicated severe deficiency but serum Cu indicated sufficient levels throughout most of the grazing season. However whole blood SOD activity decreased at d 56 and remained at this level at d 112. Due to differences in which SOD activity is reported in the literature, it is difficult to assess an absolute value of SOD activity at which Cu is considered insufficient. Paynter (1987) reported an asymptotic relationship between erythrocyte SOD activity (U/g hemoglobin) and liver Cu concentration (wet weight) in deer (*Axis porcinus*). As liver Cu decreased below 20 μg/g, SOD activity began to

decrease and at 10 µg/g liver Cu, SOD decreased severely. In 2005, the decrease in SOD activity at d 112 suggests that Cu function was being compromised, but liver and serum Cu levels indicated that Cu pools should be available to maintain SOD activity. The decrease in SOD activity may be explained by the dynamics of blood and the life span of erythrocytes. The average life span of bovine erythrocytes is 120 d (Mizuno et al., 1959). In erythrocytes, the enzyme appears to be synthesized at the time of erythropoesis, and the life span of SOD correlates to the life span of the erythrocyte (Paynter, 1987) and SOD activity is suggested to be an indicator of chronically induced cupper deficiencies. Therefore, the SOD activity may reflect the Cu status of these steers prior to the beginning of this experiment.

mRNA expression of Cu/Zn SOD

There was no treatment effect on hepatic Cu/Zn superoxide dismutase (Sod1) gene expression in both 2004 (P = 0.33; Figure 5-3) or 2005 (P = 0.92; Figure 5-4). Bhusari et al. (2006) and Settivari et al. (2006) reported microarray analysis of hepatic tissue from mice consuming E+ and E- diets. These authors indicated no changes in hepatic Sod1 expression. Due to biotransformation of alkaloids in the rumen, the form in which toxins reach the liver are potentially different and therefore will affect hepatic tissue differently compared to monogastrics. The similarities in the results of current research and that of Bhusari et al. (2006) and Settivari et al. (2006) indicate that the consumption of an E+ diet does not affect the expression of Sod1, independent of dietary Cu.

During both 2004 and 2005 there was an effect of sampling date ($P < 0.05$) on expression of Sod1. In 2004 Sod1 expression was highest on d 0 and similar between d 56 and 112. This pattern of Sod1 expression was similar to that of SOD activity. In 2005, Sod1 expression was highest early in the season (d 21) and decreased to d 56 and

although not significantly different, slightly increased on d 112. The greater expression on d 21 compared to the rest of the season is likely due to increased stress associated with transportation and new environment within the first 21 d. These trends are similar to that of SOD activity on d 21 and 112. On d 56, Sod1 expression decreased while SOD activity remained elevated, which may be explained by the relationship of erythropoesis and the incorporation of SOD described earlier.

Transcription of the Sod1 gene is dependent on the activation of Cu-dependent transcription factor protein ACE1 that binds to a single location of the promoter region of the Sod1 gene in yeast (Thiele, 1992). The proposed physiological reasons for this Cu-dependent transcription in yeast include: 1) an intracellular signal of substrate necessary to produce Sod1 and 2) since Cu increases intercellular free radicals through interactions with O_2^-, a substrate for SOD, Sod1 transcription is induced by Cu-dependent ACE1 to lower the levels of reactive O_2^- (Gralla et al., 1991). In 2005, Sod1 expression and serum Cu followed similar patterns on days Sod1 expression was measured. However, due to the sparing effect of SOD activity compared to serum Cu (Suttle and McMurray, 1983), and this pattern not evident in 2004, these similarities in pattern are likely coincidental. In addition, in 2005 liver Cu levels were either sufficient or only slightly deficient at the sampling dates suggesting Sod1 expression should not be compromised.

CONCLUSIONS

These data demonstrate that cattle grazing E+ tall fescue have reduced Cu intake compared to those grazing E- tall fescue contributing to lower liver Cu after 112 d of grazing. At the level of DMI in this experiment, steers grazing all forages were not able to increase liver Cu, but were able to maintain liver Cu. This suggests that the threshold at which dietary Cu intake negatively affects liver Cu was not attained in this research.

Also, Cu deficiency exhibited by steers grazing E+ and other forages was not great enough to compromise SOD status.

TABLES AND FIGURES

Table 5-1 Primer sequence for Real-Time PCR.

Gene name	Primer Sequence
SOD1 (Forward)	CGGTGGGCCAAAAGATGA
SOD1 (Reverse)	CACCGTTTTTGTCAGCTGTCA
GAPDH (Forward)	GCATCGTGGAGGGACTTATGA
GAPDH (Reverse)	GGGCCATCCACAGTCTTCTG

Table 5-2. Least squares means of forage Cu and Zn from Kentucky 31 endophyte infected and endophye free, Q4508-AR542 non-ergot alkaloid-producing endophyte infected tall fescues, and Lakota prairie grass pastures in 2004.

	Treatment[1]				
Mineral/Year	E-	Q	E+	L	SE
	----------μg/g----------				
Cu					
2004	4.0[b]	3.9[bc]	3.7[c]	4.9[a]	0.09
2005	4.9	5.0	4.6		0.12
Avg	4.4	4.5	4.2		0.86
Zn					
2004	20.2[a]	18.6[b]	17.3[c]	19.8[b]	0.36
2005	16.2	17.3	16.0		0.44
Avg	18.2	17.9	16.6		0.69

[1]Treatments : E-= Kentucky 31 endophyte free tall fescue, E+ = Kentucky 31 endophyte infected tall fescue, Q = Q4508 AR542 non-ergot alkaloid-producing endophyte infected fescue, L = Lakota prairie grass.

[abc] Within a year, means without a common superscript letter differ, $P < 0.05$.

Table 5-3. Least squares means of seasonal forage Cu from Kentucky 31 endophyte infected and endophye free, Q4508-AR542 non-ergot alkaloid-producing endophyte infected tall fescues, and Lakota prairie grass pastures

	Treatment[1]				
Item/Year	E-	Q	E+	L	SE
	----------µg/g----------				
2004					
May 28	3.9	3.8	3.5	4.3	0.48
June 24	2.3[b]	2.8[ab]	2.2[b]	3.4[a]	0.26
July 23	3.4[b]	3.1[b]	3.0[b]	4.4[a]	0.23
August 20	3.9[b]	4.0[b]	3.4[b]	4.9[a]	0.19
September 17	6.5	5.9	6.5	7.5	0.43
2005					
May 31	2.6	2.7	2.5		0.53
June 28	3.7	3.4	4.0		0.20
July 26	5.6	5.4	5.8		0.32
August 23	5.9	6.0	6.5		0.37
September 16	6.6	5.9	6.3		0.20

[1]Treatments : E-= Kentucky 31 endophyte free tall fescue, E+ = Kentucky 31 endophyte infected tall fescue, Q = Q4508 AR542 non-ergot alkaloid-producing endophyte infected fescue, L = Lakota prairie grass.

[ab] Within a row, means without a common superscript letter differ, $P < 0.05$.

Table 5-4. Least squares means of seasonal Cu intake of steers grazing Kentucky 31 endophyte infected and endophye free, Q4508-AR542 non-ergot alkaloid-producing endophyte infected tall fescues, and Lakota prairie grass pastures

	Treatment[1]			
Year/Period[2]	E-	Q	E+	L
	----------µg Cu/kg DM----------			
2004[3]				
Period 1	157.8[a]	93.3[c]	114.0[b]	95.3[c]
Period 2	166.5[a]	85.5[c]	64.4[d]	109.1[b]
Period 3	89.2[c]	70.8[d]	59.7[d]	114.2[b]
SE	0.43	0.37	0.35	0.35
2005[4]				
Period 1	132.9[bcd]	122.8[de]	91.9[e]	
Period 2	118.8[de]	128.3[de]	131.5[cd]	
Period 3	177.7[a]	150.4[abc]	163.5[ab]	
SE	1.37	1.36	1.36	

[1]Treatments : E-= Kentucky 31 endophyte free tall fescue, E+ = Kentucky 31 endophyte infected tall fescue, Q = Q4508 AR542 non-ergot alkaloid-producing endophyte infected fescue, L = Lakota prairie grass.
[2] Period: 2004, 1 = May 26 to June 1; 2 = July 7 to 13; 3 = September 1 to 7; 2005, 1 = June 13 to 19; 2 = Aug 1 to 7; 3 = September 20-26.
[3]Treatment x period interaction, $P < 0.001$.
[4]Treatment x period interaction, $P < 0.01$.
[abcde]Within a year, means without a common superscript letter differ, $P < 0.05$.

Table 5-5. Least squares means of serum Cu for steers grazing Kentucky 31 endophyte infected and endophye free, Q4508-AR542 non-ergot alkaloid-producing endophyte infected tall fescues, and Lakota prairie grass pastures

Treatment[1]	2004	2005	Avg
	----------µg/dL----------		
E-	0.67	0.67[a]	0.67
Q	0.67	0.71[a]	0.69
E+	0.68	0.59[b]	0.63
L	0.72		
S.E.	0.05	0.02	0.02

[1]Treatments : E-= Kentucky 31 endophyte free tall fescue, E+ = Kentucky 31 endophyte infected tall fescue, Q = Q4508 AR542 non-ergot alkaloid-producing endophyte infected fescue, L = Lakota prairie grass.

[ab]Within a column, means without a common superscript letter differ, $P < 0.05$.

Table 5-6. Least squares means of seasonal serum Cu from steers grazing Kentucky 31 endophyte infected and endophye free, Q4508-AR542 non-ergot alkaloid-producing endophyte infected tall fescues, and Lakota prairie grass pastures

	Treatment[1]				
Item/Year	E-	Q	E+	L	SE
	----------µg/dL----------				
2004					
May 5	0.76	0.77	0.85	0.85	0.06
May 28	0.69	0.75	0.70	0.78	0.04
June 24	0.76	0.83	0.74	0.78	0.03
July 23	0.56	0.54	0.53	0.58	0.03
August 20	0.57	0.60	0.57	0.64	0.03
September 17	0.70	0.64	0.69	0.69	0.01
2005					
May 3	0.67	0.68	0.50		0.05
May 31	0.78	0.82	0.74		0.07
June 28	0.72[ab]	0.82[a]	0.70[a]		0.03
July 26	0.50	0.53	0.45		0.04
August 23	0.62[a]	0.66[a]	0.54[b]		0.01
September 16	0.77[a]	0.74[a]	0.61[b]		0.03

[1]Treatments : E-= Kentucky 31 endophyte free tall fescue, E+ = Kentucky 31 endophyte infected tall fescue, Q = Q4508 AR542 non-ergot alkaloid-producing endophyte infected fescue, L = Lakota prairie grass.

[ab] Within a row, means without a common superscript letter differ, $P < 0.05$.

Table 5-7. Least squares means[1] of seasonal Cu liver (wet weight) in animals grazing of from steers grazing Kentucky 31 endophyte infected and endophye free, Q4508-AR542 non-ergot alkaloid-producing endophyte infected tall fescues, and Lakota prairie grass pastures

Year/Sampling Day	Treatment[2]							
	E-	SE	Q	SE	E+	SE	L	SE
	----------µg/g----------							
2004								
d-0	9.8		18.0		10.6		17.1	
d-56	12.3	1.8	12.9	1.7	11.6	1.6	13.5	1.7
d-112	19.2[a]	1.0	16.7[ab]	1.0	11.9[c]	1.0	13.4[bc]	1.0
Avg	15.9[c]	0.9	14. 8[cd]	0.9	11.7[d]	0.9	13.4[cd]	0.9
2005								
d-0	31.9		19.5		16.6			
d-56	28.0	3.7	26.3	2.8	33.4	3.2		
d-112	26.2	3.8	23.3	2.9	24.4	3.2		
Avg	27.1	3.5	24.8	2.7	28.9	3.0		

[1] Least squares means are adjusted using d 0 as a covariate for liver Cu.
[2] Treatments : E-= Kentucky 31 endophyte free tall fescue, E+ = Kentucky 31 endophyte infected tall fescue, Q = Quantum AR542 non-toxic endophyte infected fescue, L = Lakota prairie grass.
[abc] Within a row, means without a common superscript letter differ, $P < 0.05$.

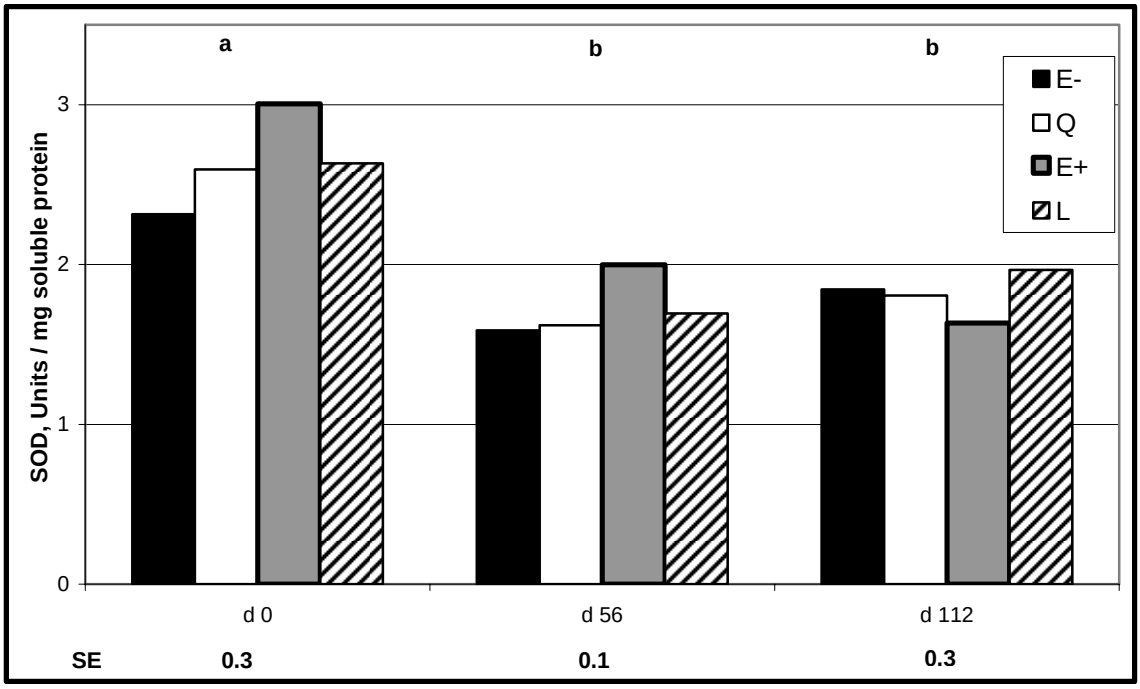


Figure 5-1. Whole blood Cu/Zn superoxide dismutase (SOD) activity of steers grazing Kentucky 31 endophyte infected (E+) and endophye free (E-), and Q4508-AR542 non-ergot alkaloid-producing endophyte infected tall fescues (Q) tall fescues and 'Lakota' prairie grass (L) pastures in 2004. There were no differences among treatments on d 0 (P = 0.60), d 56 (P = 0.31), or d 112 (P = 0.92). [ab]Sampling dates with different superscript differed at $P < 0.05$ (SE = 146).

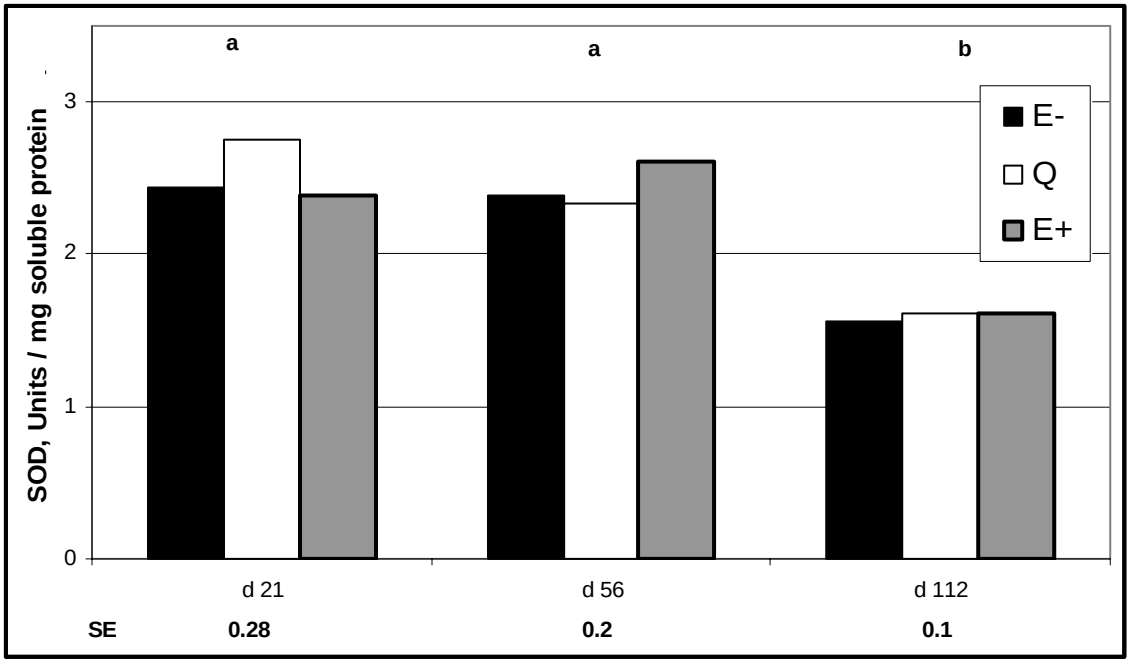


Figure 5-2. Whole blood Cu/Zn superoxide dismutase (SOD) activity of steers grazing Kentucky 31 endophyte infected (E+) and endophye free (E-), and Q4508-AR542 non-ergot alkaloid-producing endophyte infected tall fescues (Q) tall fescues pastures in 2005. There were no differences among treatments on d 21 (P = 0.64), d 56 (P = 0.77), or d -112 (P = 0.93). [ab]Sampling dates with different superscript differed at $P < 0.05$ (SE = 110).

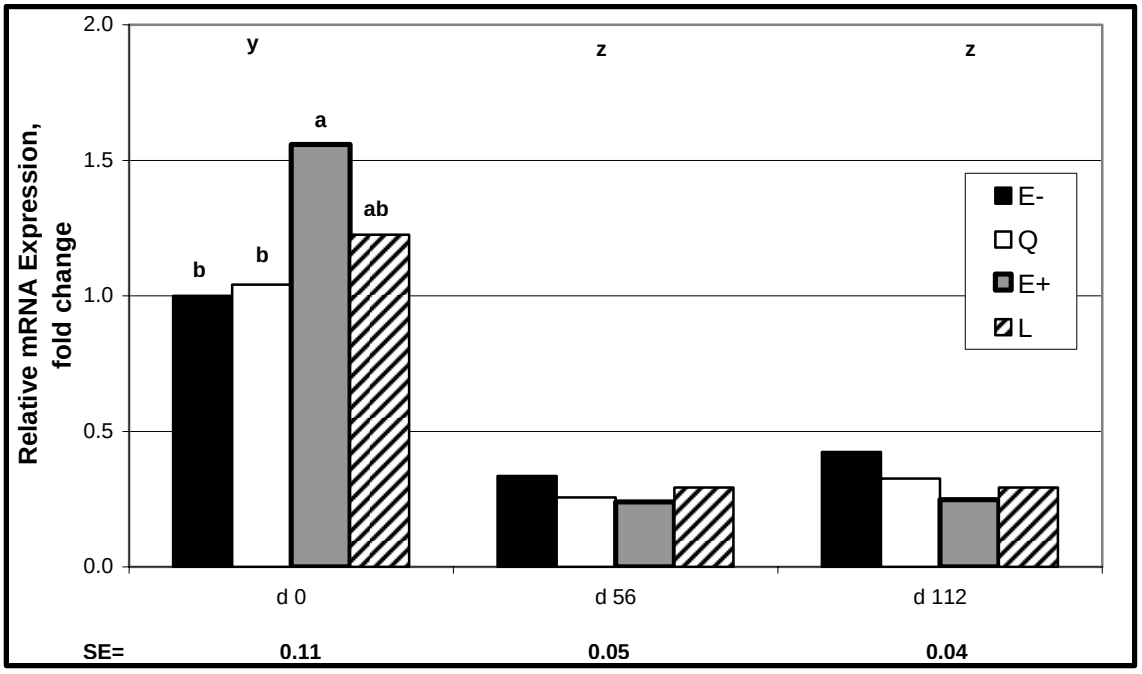


Figure 5-3. Hepatic Sod1 relative quantification of steers grazing Kentucky 31 endophyte infected (E+) and endophye free (E-), and Q4508-AR542 non-ergot alkaloid-producing endophyte infected tall fescues (Q) tall fescues and 'Lakota' prairie grass (L) pastures in 2004. There were no differences among treatments on d 56 (P = 0.56) or d 112 (P = 0.45). [ab] Treatments on d-0 with different script differed at $P < 0.05$ (SE = 0.05). [yz]Sampling dates with different subscript differed at $P < 0.05$ (SE = 0.06).

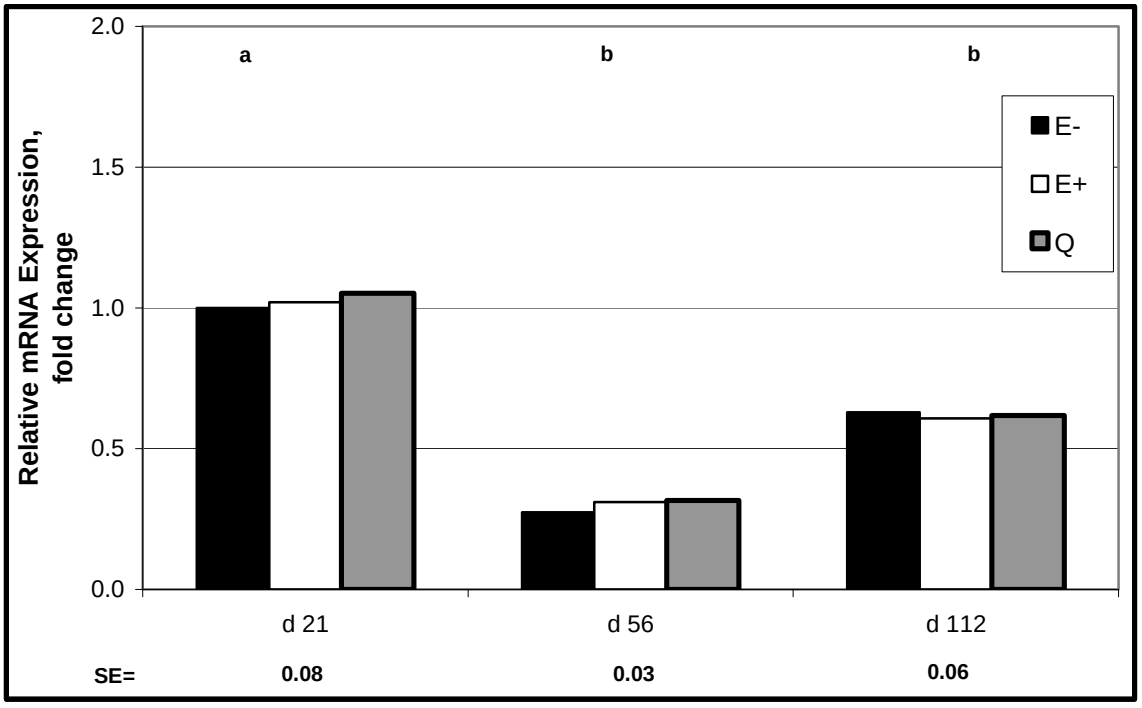


Figure 5-4. Hepatic Sod1 relative quantification of steers grazing Kentucky 31 endophyte infected (E+) and endophye free (E-), and Q4508-AR542 non-ergot alkaloid-producing endophyte infected tall fescues (Q) tall fescues pastures in 2005. There were no differences among treatments on d 21 (P = 0.90), d 56 (P =0.49), or d 112 (P = 0.49). [ab]Sampling dates with different subscript differed at $P < 0.05$ (SE = 0.04).

LITERATURE CITED

Bhusari, S., L. B. Hearne, D. E. Spiers, W. R. Lamberson, and E. Antoniou. 2006. Effect of fescue toxicosis on hepatic gene expression in mice. J. Anim. Sci. 84: 1600-1612.

Coffey, K. P., J. L. Moyer, L. W. Lomas, J. E. Smith, D. C. La Rue, and F. K. Brazle. 1992. Implant and copper oxide needles for steers grazing Acremonium coenophialum-infected tall fescue pastures: effects on grazing and subsequent feedlot performance and serum constituents. J. Anim. Sci. 70: 3203-3214.

Corah, L. R., and D. Dargatz. 1996. Forage analyses from cow/calf herds in 18 states. Cow/Calf Health and Prod Audit. USDA. APHIS.

Cousins, R. J. 1985. Absorption, transport and hepatic metabolism of copper and zinc with special reference to metallothionein and caeruloplasmin. Physiol. Rev. 65: 238-309.

Dennis, S. B., V. G. Allen, K. E. Saker, J. P. Fontenot, J. Y. Ayad, and C. P. Brown. 1998. Influence of Neotyphodium coenophialum on copper concentration in tall fescue. J. Anim Sci. 76: 2687-2693.

Donohue, S. J. e. 1992. Reference Soil and Media Diagnostic Procedures for the Southern Region of the United States Southern Coop. Ser. Bull. No. 374 VA Ag. Exp. Stat., Virginia, Tech, Blacksburg, VA.

Gengelbach, G. P., J. D. Ward, J. W. Spears, and T. T. Brown, Jr. 1997. Effects of copper deficiency and copper deficiency coupled with high dietary iron or molybdenum on phagocytic cell function and response of calves to a respiratory disease challenge. J. Anim. Sci. 75: 1112-1118.

Gornall, A. G., C. J. Bardawill, and M. M. David. 1949. Determination of proteins by means of the biuret reaction. J. Biol. Chem. 177: 751-766.

Gralla, E. B., D. J. Thiele, P. Silar, and J. S. Valentine. 1991. ACE1, a copper-dependent transcription factor, activates expression of the yeast copper, zinc superoxide dismutase gene. Proc. Nat. Acad. Sci. 88: 8558-8562.

Grings, E. E., and W. W. Poland. 2000. Effect of hay source and level on tissue trace mineral concentrations in growing steers. J. Anim. Sci. 78(Suppl. 2): 114 (Abstr.).

Halliwell, B. 1987. Oxidants and human disease: some new concepts. FASEB J. 1: 358-364.

Hoveland, C. S. 2003. The fescue toxicosis story: an update. 35th Beef Improvement Federation Meeting, Lexington, KY.

LaCasha, P. A., H. A. Brady, V. G. Allen, C. R. Richardson, and K. R. Pond. 1999. Voluntary intake, digestibility, and subsequent selection of Matua bromegrass, coastal bermudagrass, and alfalfa hays by yearling horses. J. Anim. Sci. 77: 2766-2773.

Livak, K. J., and T. D. Schmittgen. 2001. Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2-ΔΔCt Method. Methods 25: 402-408.

Malinowski, D., H. Zuo, D. Belesky, and G. Alloush. 2004. Evidence for copper binding by extracellular root exudates of tall fescue but not perennial ryegrass infected with *Neotyphodium* spp. endophytes. Plant and Soil 267: 1-12.

McCord, J. M., and I. Fridovich. 1969. Superoxide dismutase: an enzymatic function for erythrocuprein. J. Biol. Chem. 244: 6049-6055.

Mizuno, N. S., V. Perman, F. W. Bates, J. H. Sautter, and M. O. Schultze. 1959. Life Span of Thrombocytes and Erythrocytes in Normal and Thrombocytopenic Calves. Blood 14: 708-719.

Muchovej, R. M., V. G. Allen, D. C. Martens, L. W. Zelazny, and D. R. Notter. 1986. Aluminum, citric acid, nitriloacetic acid, and soil moisture effects on aluminum and iron concentration in ryegrass. Agron. J. 78: 138-145.

NRC. 2000. Nutrient Requirements of Beef Cattle. 7th ed. Natl. Acad Press, Washington, D.C.

Paynter, D. J. 1987. The diagnosis of copper insufficiency. In: J. M. Howell and J. M. Gawthorne (eds.) Copper in Animals and Man. Vol. 1. p 101-119. CRC Press, Boca Raton, FL.

Puschner, B., Y. K. Choi, J. H. Tegzes, and M. C. Thurmond. 2004. Influence of age, sex, and production class on liver zinc concentration in calves. J. Vet. Diagn. Invest. 16: 278-282.

Saker, K. E., V. G. Allen, J. Kalnitsky, C. D. Thatcher, W. S. Swecker, Jr., and J. P. Fontenot. 1998. Monocyte immune cell response and copper status in beef steers that grazed endophyte-infected tall fescue. J. Anim Sci. 76: 2694-2700.

Settivari, R. S., S. Bhusari, T. Evans, P. A. Eichen, L. B. Hearne, E. Antoniou, and D. E. Spiers. 2006. Genomic analysis of the impact of fescue toxicosis on hepatic function. J. Anim. Sci. 84: 1279-.

Suttle, N. F., and C. H. McMurray. 1983. Use of erythrocyte coper:zinc superoxide dismutase activity and hair or fleece concentrations in the diagnosis of hypocuprosis. Res. Vet. Sci. 35: 47-52.

Thiele, D. J. 1992. Metal-regulated transcription in eukaryotes. Nucleic Acids Res. 20: 1183-1191.

Uauy, R., M. Olivares, and M. Gonzalez. 1998. Essentiality of copper in humans. Am. J. Clin. Nutr. 67(suppl): 952S-959S.

Underwood, E. J., and N. F. Shuttle. 1999. The Mineral Nutrition of Livestock. 3rd ed. CAB Int., New York, NY.

Ward, J. D., and J. W. Spears. 1997. Long-term effects of consumption of low-copper diets with or without supplemental molybdenum on copper status, performance, and carcass characteristics of cattle. J. Anim. Sci. 75: 3057-3065.

Ward, J. D., J. W. Spears, and E. B. Kegley. 1993. Effect of copper level and source (copper lysine vs copper sulfate) on copper status, performance, and immune response in growing steers fed diets with or without supplemental molybdenum and sulfur. J. Anim. Sci. 71: 2748-2755.

Wikse, S. E., D. Herd, R. Field, and P. Holland. 1992. Diagnosis of copper deficiency in cattle. J. Am. Vet. Med. Assoc. 200: 1625-1629.

CHAPTER 6
CONCLUSIONS

Tall fescue continues to be an important forage resource for the beef cattle industry despite the deleterious effects of fescue toxicosis on animal performance. The data from these experiments demonstrate that steers consuming E+ suffer from fescue toxicosis. Gains of steers grazing E- were greater than those grazing E+ which is commonly reported in the literature. Similarly, steers grazing Q and L also gained more compared to those grazing E+, however caution should be used when considering these forages as replacements or compliments to E+. Previous research has shown non-ergot alkaloid-producing endophyte-infected fescues produce gains superior to E+, but in the current research Q produced gains lower than E-. This suggests that Q4508 is not the optimal fescue cultivar for the incorporation of these non-toxic endophytes. In addition, Lakota may not be a practical choice due to the level of management needed to ensure persistence of this forage. Higher levels of N needed to produce these levels of gain and intolerance to heavy grazing make the use of this forage unfeasible for many beef cattle producers.

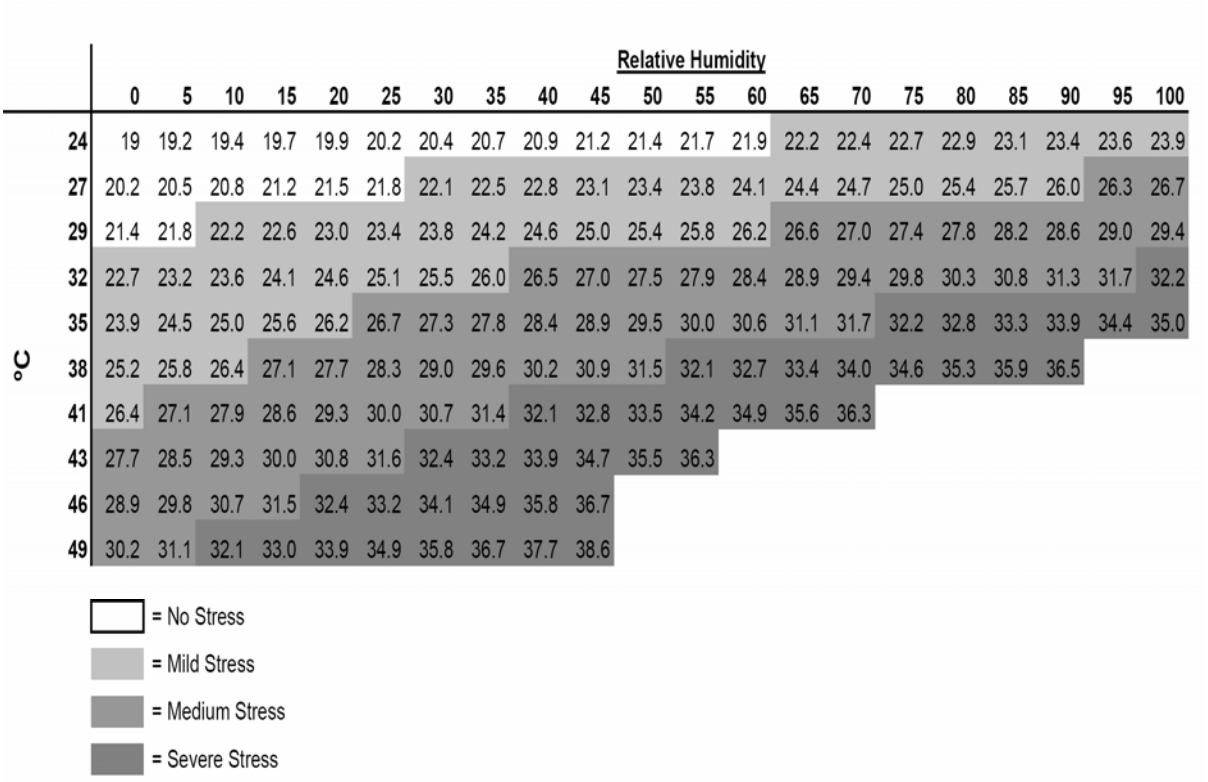
This is the first report of the presence of LSA in the ruminal fluid of steers grazing endophyte-infected tall fescue. The presence of LSA suggests it is available for transport across the ruminal wall and may contribute to fescue toxicosis. This supports the previous suggestion that simple ergoline alkaloids are the causative agent in fescue toxicosis.

Forage Cu levels were below the NRC requirement of Cu for growing cattle in all forages and when decreased liver Cu status of steers grazing E+ was observed, DMI was lower on E+ compared to steers grazing E-. This suggests that decreased DMI can be influential in the Cu status of animals grazing E+ tall fescue. However, at these low levels of Cu intake in all treatments, steers were able to maintain liver Cu levels. These data indicate that it is essential for producers to assess the Cu status of their herd and/or incoming stocker cattle. In the case of Cu deficient animals, Cu supplementation is critical as forage alone will not increase liver Cu to sufficient levels. The similarities of SOD status of steers grazing E+ or other forages indicate that the differences observed in liver Cu were not enough to compromise its function. This suggests that the threshold of Cu intake at which Cu depletion occurs was not reached under these experimental conditions. Also, the similarities in SOD status among treatments suggests that oxidative stress is not contributing to fescue toxicosis.

This research also indicates the need for further research in different areas. Further research in the metabolism and bioavailability of LSA and other alkaloids in the rumen and other tissues of grazing animals is needed to determine the exact alkaloid(s) responsible for fescue toxicosis. Once the compounds are identified, possible pharmacological strategies can be explored to allow livestock producers to continue to utilize E+ tall fescue while eliminating the effects of fescue toxicosis on production.

APPENDIX 1

TEMPERATURE HUMIDITY INDEX (THI) FOR CATTLE.



°C \ Relative Humidity	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100
24	19	19.2	19.4	19.7	19.9	20.2	20.4	20.7	20.9	21.2	21.4	21.7	21.9	22.2	22.4	22.7	22.9	23.1	23.4	23.6	23.9
27	20.2	20.5	20.8	21.2	21.5	21.8	22.1	22.5	22.8	23.1	23.4	23.8	24.1	24.4	24.7	25.0	25.4	25.7	26.0	26.3	26.7
29	21.4	21.8	22.2	22.6	23.0	23.4	23.8	24.2	24.6	25.0	25.4	25.8	26.2	26.6	27.0	27.4	27.8	28.2	28.6	29.0	29.4
32	22.7	23.2	23.6	24.1	24.6	25.1	25.5	26.0	26.5	27.0	27.5	27.9	28.4	28.9	29.4	29.8	30.3	30.8	31.3	31.7	32.2
35	23.9	24.5	25.0	25.6	26.2	26.7	27.3	27.8	28.4	28.9	29.5	30.0	30.6	31.1	31.7	32.2	32.8	33.3	33.9	34.4	35.0
38	25.2	25.8	26.4	27.1	27.7	28.3	29.0	29.6	30.2	30.9	31.5	32.1	32.7	33.4	34.0	34.6	35.3	35.9	36.5		
41	26.4	27.1	27.9	28.6	29.3	30.0	30.7	31.4	32.1	32.8	33.5	34.2	34.9	35.6	36.3						
43	27.7	28.5	29.3	30.0	30.8	31.6	32.4	33.2	33.9	34.7	35.5	36.3									
46	28.9	29.8	30.7	31.5	32.4	33.2	34.1	34.9	35.8	36.7											
49	30.2	31.1	32.1	33.0	33.9	34.9	35.8	36.7	37.7	38.6											

Modified from Dr. Frank Wierama (1990), Department of Agriculture Engineering, The University of Arizona, Tuscon, Arizona.

LIST OF REFERENCES

Abaye, A. O., J. F. Guay, P. Peterson, V. Mulkey, S. Hutton, and R. Smith. 2002. 'Matua' Prairie Grass (*Bromus willdenowii*). Va. Coop. Ext. New Forage Pub. 424-700. Blacksburg, Va.

Adams, D. C., R. E. Short, M. M. Borman, and M. D. MacNeil. 1991. Estimation of fecal output with an intra-ruminal continuous release marker device. J. Range Manag. 44: 204-207.

Adcock, R. A., N. S. Hill, J. H. Bouton, H. R. Boerma, and G. O. Ware. 1997. Symbiont regulation and reducing ergot alkaloid concentration by breeding endophyte-infected tall fescue. J. Chem. Ecol. 23: 691-704

Agee, C. S., and N. S. Hill. 1994. Ergovaline variability on *Acremonium* infected tall fescue due to environment and plant genotype. Crop Sci. 34: 221-226.

Al-Haidary, A., D. E. Spiers, G. E. Rottinghaus, G. B. Garner, and M. R. Ellersieck. 2001. Thermoregulatory ability of beef heifers following intake of endophyte-infected tall fescue during controlled heat challenge. J. Anim. Sci. 79: 1780-1788.

Aldrich, C. G., M. T. Rhodes, J. L. Miner, M. S. Kerley, and J. A. Paterson. 1993. The effects of endophyte-infected tall fescue consumption and use of a dopamine antagonist on intake, digestibility, body temperature, and blood constituents in sheep. J. Anim. Sci. 71: 158-163.

Alloway, B. J. 1973. Copper and molybdenum in swayback pastures. J. Agric. Sci. (Cambridge) 80: 521-524.

AOAC. 2000. Official Methods of Analysis (17th Ed.). Association of Official Analytical Chemists, Washington, DC.

Arechavaleta, M., C. W. Bacon, R. D. Plattner, C. S. Hoveland, and D. E. Radcliffe. 1992. Accumulation of Ergopeptide Alkaloids in Symbiotic Tall Fescue Grown under Deficits of Soil Water and Nitrogen Fertilizer. Appl. Environ. Microbiol. 58: 857-861.

Bacon, C. W., J. K. Porter, J. D. Robbins, and E. S. Luttrell. 1977. *Epichloe typhina* from tall fescue grasses. Appl. Environ. Microbiol. 35: 576-581.

Bacon, C. W., and M. R. Siegel. 1988. Endophyte parasitism of tall fescue. J. Prod. Agric. 1: 45-55.

Bazer, F. M. 1992. Mediators of maternal recognition of pregnancy in mammals. Proc. Soc. Exp. Biol. Med. 199: 373-384.

Becker, R. B., J. R. Henderson, and R. B. Leighty. 1965. Mineral malnutrition in cattle. Florida Agr. Expt. Sta. Bull. 699.

Berdanier, C. D. 1994. Trace Minerals In: Advanced Nutrition Micronutrients. p 200-203. CRC Press, New York.

Berry, N. R., M. R. L. Scheeder, F. Sutter, T. F. Krober, and M. Kreuzer. 2000. The accuracy of intake estimation based on the use of alkane controlled-release capsules and faeces grab sampling in cows. Ann. Zootech 49: 3-13.

Bhusari, S., L. B. Hearne, D. E. Spiers, W. R. Lamberson, and E. Antoniou. 2006. Effect of fescue toxicosis on hepatic gene expression in mice. J. Anim. Sci. 84: 1600-1612.

Blankenship, J. D., M. J. Spiering, H. H. Wilkinson, F. F. Fannin, L. P. Bush, and C. L. Schardl. 2001. Production of loline alkaloids by the grass endophyte, Neotyphodium uncinatum, in defined media. Phytochemistry 58: 395-401.

Bouton, J. H., G. C. M. Latch, N. S. Hill, C. S. Hoveland, M. A. McCann, R. H. Watson, J. A. Parish, L. L. Hawkins, and F. N. Thompson. 2002. Reinfection of tall fescue cultivars with non-ergot alkaloid-producing endophytes. Agron. J. 94: 567-574.

Bransby, D. I. 1997. Steer weight gain responses to ivermectin when grazing fescue. Large Anim. Pract. 18: 16-19.

Bransby, D. I., S. P. Schmidt, W. Griffey, and J. T. Eason. 1988. Heavy grazing is best for infected fescue. Alabama Agric. Exp. Stn. Highlights Agric. Res. 35: 12.

Brazle, F. K., and K. P. Coffey. 1991. Effect of zeranol on performance of steers grazing high-and low-endophyte tall fescue pastures. Prof. Anim. Sci. 7: 39-42.

Browning, R., Jr., and M. L. Leite-Browning. 1997. Effect of ergotamine and ergonovine on thermal regulation and cardiovascular function in cattle. J. Anim. Sci. 75: 176-181.

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

Buckner, R. C., J. B. Powell, and R. V. Frakes. 1979. Historic development. In: R. C. Buckner and L. P. Bush (eds.) Tall Fescue. p 1-8. Amer. Soc. Agron., Madison, WI.

Buntinx, S. E., K. R. Pond, D. S. Fisher, and J. C. Burns. 1992. Evaluation of the Captec chrome controlled-release device for the estimation of fecal output by grazing sheep. J. Anim. Sci. 70: 2243-2249.

Burdine, K. H., and R. Trimble. 2005. The economics of replacing endophyte infected fescue. Ext. Pub. No. 2005-01C. University of Kentucky. available online: http://www.uky.edu/Ag/AgEcon/pubs/ext_aec/ext2005-01c.html: Accessed: November 13, 2005.

Burke, J. M., D. E. Spiers, F. N. Kojima, G. A. Perry, B. E. Salfen, S. L. Wood, D. J. Patterson, M. F. Smith, M. C. Lucy, W. G. Jackson, and E. L. Piper. 2001. Interaction of endophyte-infected fescue and heat stress on ovarian function in the feef heifer. Biol. Reprod. 65: 260-268.

Burns, J. C., K. R. Pond, and D. S. Fisher. 1991. Effects of grass species on grazing steers. II. Dry matter intake and digesta kinetics. J. Anim. Sci. 69: 1199-1204.

Burns, J. C., K. R. Pond, and D. S. Fisher. 1994. Measurement of forage intake. In: J. G.C. Fahey, M. Collins, D. R. Mertens and L. E. Moser (eds.) Forage Quality, Evaluation, and Utilization. p 494-532. Am. Soc. of Agro.n Inc.; Crop Sci. Soc. Am. Inc.; Soil Sci. Soc. Am. Inc., Madison, WI, USA.

Burns, J. C., and L. E. Sollenberger. 2002. Grazing behavior of ruminants and daily performance from warm-season grasses. Crop Sci. 42: 873-881.

Chestnut, A. B., H. A. Fribourg, J. B. McLaren, D. G. Keltner, B. B. Reddick, R. J. Carlisle, and M. C. Smith. 1991. Effects of *Acremonium coenophialum* infestation, bermudagrass, and nitrogen or clover onsteers grazing tall fescue pastures. J. Prod. Agric. 4: 208-213.

Clay, K. 1988. Fungal endophytes of grasses: A defensive mutualism between plants and fungi. Ecology 69: 10-16.

Coblentz, W. K., K. P. Coffey, J. E. Turner, D. A. Scarbrough, J. S. Weyers, K. F. Harrison, Z. B. Johnson, L. B. Daniels, C. F. Rosenkrans, Jr., D. W. Kellog, and D. S. Hubbell, III. 2000. Effect of maturity on degradation kinetics of sod-seeded cereal grain forage grown in northern Arkansas. J. Dairy Sci. 83: 2499-2511.

Coleman, S. W., and K. M. Barth. 1973. Quality of diets selected by grazing animals and its relation to quality of available forage and species composition of pastures. J. Anim. Sci. 36: 754-761.

Conrad, H. R., A. D. Pratt, and J. W. Hibbs. 1964. Regulation of feed intake in dairy cows. I Change in importance of physical and physiological factors with increasing digestibility. J. Dairy Sci. 47: 54-62.

Corah, L. R., and D. Dargatz. 1996. Forage analyses from cow/calf herds in 18 states. Cow/Calf Health and Prod Audit. USDA. APHIS.

Cousins, R. J. 1985. Absorption, transport and hepatic metabolism of copper and zinc with special reference to metallothionein and caeruloplasmin. Physiol. Rev. 65: 238-309.

Crampton, E. W., E. Donefer, and L. E. Lloyd. 1960. A nutritive value index of forages. J. Anim. Sci. 19: 538-544.

Craven, K. D., K. Clay, and C. L. Schardl. 2005. Systematics and morphology. In: H. A. Fribourg and D. B. Hannaway (eds.) Tall Fescue-on-line monograph. Oregon State University, Corvalas, OR. http://forages.oregonstate.edu/is/tfis/book.cfm?PageID=369.

Cross, D. L., K. Anas, W. C. Bridges, and J. H. Chappell. 1999. Clinical effects of domperidone on fescue toxicosis in pregnant mares. Proc. Am. Assoc. Equine Pract. 45: 203-206.

Davenport, G. M., J. A. Boling, and C. H. Rahe. 1993. Growth and endocrine responses of cattle to implantation of estradiol-17B during continuous or discontinuous grazing of high- and low-endophyte-infected tall fescue. J. Anim. Sci. 71: 757-764.

De Vries, M. F. W. 1995. Estimating forage intake and quality in grazing cattle: A reconsideration of the hand-plucking method. J. Range Manag. 48: 370-375.

Dennis, S. B., V. G. Allen, K. E. Saker, J. P. Fontenot, J. Y. Ayad, and C. P. Brown. 1998. Influence of Neotyphodium coenophialum on copper concentration in tall fescue. J. Anim Sci. 76: 2687-2693.

Donohue, S. J. e. 1992. Reference Soil and Media Diagnostic Procedures for the Southern Region of the United States Southern Coop. Ser. Bull. No. 374 VA Ag. Exp. Stat., Virginia, Tech, Blacksburg, VA.

Dougherty, C. T., N. W. Bradley, L. M. Lauriault, J. E. Arias, and P. L. Cornelius. 1992. Allowance-intake relations of cattle grazing vegetative tall fescue. Grass and Forage Sci. 47: 211-219.

Dove, H. 1991. Using the n-alkanes of plant cuticular wax to estimate the species composition of herbage mixtures. Aust. J. Agric. Res. 43: 1711-1724.

Dove, H., and R. W. Mayes. 1991. The use of plant wax alkanes as marker substances in studies of the nutrition of herbivores: A Review. Aust. J. Agric. Res. 42: 913-952.

Dove, H., and R. W. Mayes. 1996. Plant wax components: A new approach to estimating intake and diet composition in herbivores. J. Nutr. 126: 13-26.

Dove, H., R. W. Mayes, C. S. Lamb, and K. L. Ellis. 2002. Factors influencing the release rate of alkanes from an intra-ruminal controlled-release device, and the resultant accuracy of intake estimation in sheep. Aust. J. Agric. Res. 53: 681-696.

Dove, H., and A. D. Moore. 1995. Using a least-squares optimisation procedure to estimate botanical composition based on the alkanes of plant cuticular wax. Aust. J. Agric. Res. 46: 1535-1544.

Dove, H., and M. Olivan. 1998. Using synthetic or beeswax alkanes for estimating supplement intake in sheep. Anim. Prod. Aust. 22: 189-192.

Dove, H., J. T. Wood, R. J. Simpson, B. J. Leury, T. A. Ciavarella, K. L. Gatford, and C. Siever-Kelly. 1999. Spray-topping annual grass pasture with glyphosphate to delay loss of feeding value during summer. III. Quantitative basis of the alkane-based procedure for estimating diet selection and herbage intake by grazing sheep. Aust. J. Agric. Res. 50: 475-485.

Dubbs, T. M., E. S. Vanzant, S. E. Kitts, R. F. Bapst, B. G. Fieser, and C. M. Howlett. 2003. Characterization of season and sampling method effects on measurement of forage quality in fescue-based pastures. J. Anim. Sci. 81: 1308-1315.

Duble, R. L. 2004. Tall Fescue. Ext. Pub. Texas A&M Univ., College Station, TX: http://aggie-horticulture.tamu.edu/plantanswers/turf/publications/tallfesc.html.

Eckert, H., J. R. Kiechel, J. Rosenthaler, R. Schmidt, and E. Schreier. 1978. Biopharmaceutical aspects: Analytical methods, pharmacokinetics, metabolism, and bioavailability. In: B. Berde and H. O. Schild (eds.) Ergot Alkaloids and Related Compounds. p 719-803. Springer-Verlag, New York.

Elizalde, J. C., J. D. Cremin, Jr., D. B. Faulkner, and N. R. Merchen. 1998. Performance and digestion by steers grazing tall fescue and supplemented with energy and protein. J. Anim. Sci. 76: 1691-1701.

Faeth, S. H., M. L. Helander, and K. T. Saikkonen. 2004. Asexual Neotyphodium endophytes in a native grass reduce competitive abilities. Ecol. Lett. 7: 304-313.

Fanchamps, A. 1978. Some compounds with hallucinogenic activity. In: B. Berde and H. O. Schild (eds.) Ergot Alkaloids and Related Compounds. p 567-614. Springer-Verlag, New York.

Fike, J. H., V. G. Allen, R. E. Schmidt, X. Zhang, J. P. Fontenot, C. P. Bagley, R. L. Ivy, R. R. Evans, R. W. Coelho, and D. B. Wester. 2001. Tasco-Forage: I. Influence of a seaweed extract on antioxidant activity in tall fescue and in ruminants. J. Anim Sci. 79: 1011-1021.

Fisher, D. S. 2001. A review of a key factors regulating vountary feed intake in ruminants. Crop Sci. 42: 1651-1655.

Forcherio, J. C., G. E. Catlett, J. A. Paterson, M. S. Kerley, and M. R. Ellersieck. 1995. Supplemental protein and energy for beef cows consuming endophyte-infected tall fescue. J. Anim. Sci. 73: 3427-3436.

Forcherio, J. C., J. A. Paterson, and M. S. Kerley. 1992. Effect of source of supplemental energy and level of rumen undegradable protein on forage intake and performance of cow-calf pairs grazing endophyte-infected tall fescue pasture. J. Anim. Sci. 70(Suppl): 182(Abstr.).

Forcherio, J. C., J. A. Paterson, and M. S. Kerley. 1993. Effects of supplemental energy or protein on rumen parameters and performance of first and second partum beef cows grazing tall fescue. J. Anim. Sci. 71(Suppl. 1): 90 (Abstr.).

France, J., M. S. Dhanoa, R. C. Siddons, J. H. M. Thornley, and D. P. Poppi. 1988. Estimating production of feces by ruminants from fecal marker concentration. J. Theor. Biol 135: 383-391.

Furst, P., S. Hu, R. Hackett, and D. Hamer. 1988. Copper activates metallothionein gene transcription by altering the conformation of a specific DNA binding protein. Cell: 705-717.

Gengelbach, G. P., J. D. Ward, J. W. Spears, and T. T. Brown, Jr. 1997. Effects of copper deficiency and copper deficiency coupled with high dietary iron or molybdenum on phagocytic cell function and response of calves to a respiratory disease challenge. J. Anim. Sci. 75: 1112-1118.

Goetsch, A. L., A. L. Jones, S. R. Stokes, K. W. Beers, and E. L. Piper. 1987. Intake, digestion, passage rate and serum prolactin in growing dairy steers fed endophyte-infected fescue with noninfected fescue, clover or wheat straw. J. Anim. Sci. 64: 1759-1768.

Goetsch, A. L., K. L. Landis, G. E. Murphy, B. L. Morrison, Z. B. Johnson, E. L. Piper, A. C. Hardin, and K. L. Hall. 1988. Supplements, parasite treatments, and growth

implants for Brahman or English crossbread steers grazing endophyte-infected or noninfected fescue in spring and fall. Prof. Anim. Sci. 4: 32-38.

Gornall, A. G., C. J. Bardawill, and M. M. David. 1949. Determination of proteins by means of the biuret reaction. J. Biol. Chem. 177: 751-766.

Grace, N. D., and D. R. Body. 1981. The possible use of long chain (C_{19}-C_{32}) fatty acids in herbage as an indigestible faecal marker. J. Agric. Sci. 97: 743-745.

Gralla, E. B., D. J. Thiele, P. Silar, and J. S. Valentine. 1991. ACE1, a copper-dependent transcription factor, activates expression of the yeast copper, zinc superoxide dismutase gene. Proc. Nat. Acad. Sci. 88: 8558-8562.

Grings, E. E., and W. W. Poland. 2000. Effect of hay source and level on tissue trace mineral concentrations in growing steers. J. Anim. Sci. 78(Suppl. 2): 114 (Abstr.).

Gwinn, K. D., H. A. Fribourg, J. C. Waller, A. m. Saxton, and M. C. Smith. 1998. Changes in *Neotyphodium coenophialum* infestation levels in tall fescue pastures due to different grazing pressures. Crop Sci. 38: 201-204.

Halliwell, B. 1987. Oxidants and human disease: some new concepts. FASEB J. 1: 358-364.

Hameleers, A., and R. W. Mayes. 1998a. The use of n-alkanes to estimate herbage inkae and diet composition by dairy cows offered a perennial ryegrass/white clover mixture. Grass Forage Sci. 53: 164-169.

Hameleers, A., and R. W. Mayes. 1998b. The use of n-alkanes to estimate supplementary grass silage intake in grazing dairy cows. J. Agric. Sci. 131: 205-209.

Hannah, S. M., J. A. Paterson, J. E. Williams, M. S. Kerley, and J. L. Miner. 1990. Effects of increasing dietary levels of endophyte-infected tall fescue seed on diet digestibility and ruminal kinetics in sheep. J. Anim. Sci. 68: 1693-1701.

Hannah, S. M., M. T. Rhodes, J. A. Paterson, M. Kerley, J. E. Williams, and K. E. Turner. 1989. Influence of energy supplementation on forage intake, digestibility and grazing time by cattle grazing tall fescue. Nutr. Rep. Int. 40: 1153-1157.

Heitschmidt, R. K., and J. W. Stuth. 1991. Grazing Management: An Ecological Perspective. Timber Press, Portland, OR.

Hemken, R. W., J. A. Boling, L. S. Bull, R. H. Hatton, R. C. Buckner, and L. P. Bush. 1981. Interactions of environmental temperature and anti-quality factors on the severity of summer fescue toxicosis. J. Anim. Sci. 52: 710.

Hiatt, E. E. r., N. S. Hill, J. H. Bouton, and C. W. Mims. 1997. Monoclonal antibodies for detection of *Neotyphodium coenophialum*. Crop Sci. 37: 1265-1269.

Hill, N. S., W. C. Stringer, G. E. Rottinghaus, D. P. Belesky, W. A. Parrot, and D. D. Pope. 1990. Growth, morphological, and chemical component responses of tall fescue to *Acremonium coenophialum*. Crop Sci. 30: 156-160.

Hill, N. S., F. N. Thompson, J. A. Stuedemann, D. L. Dawe, and E. E. Hiatt, 3rd. 2000. Urinary alkaloid excretion as a diagnostic tool for fescue toxicosis in cattle. J. Vet. Diagn. Invest. 12: 210-217.

Hill, N. S., F. N. Thompson, J. A. Stuedemann, G. W. Rottinghaus, H. J. Ju, D. L. Dawe, and E. E. r. Hiatt. 2001. Ergot alkaloid transport across ruminant gastric tissues. J. Anim. Sci. 79: 542-549.

Hodgson, J. 1982. Ingestive behavior. In: J. D. Leaver (ed.) Herbage intake handbook. British Grassland Society, Hurley, Maidenhead, Berkshire.

Hoveland, C. S. 1993. Importance and economic significance of the Acremonium endophytes to performance of animals and grass plant. In: R. Joost and S. Quisenberry (eds.) Acremonium/Grass Interactions. p 3-12. Elsevier Science, Amsterdam, The Netherlands.

Hoveland, C. S. 2003. The fescue toxicosis story: an update. 35th Beef Improvement Federation Meeting, Lexington, KY.

Hoveland, C. S., R. L. Haaland, C. D. Berry, J. F. Pedersen, S. P. Schmidt, and R. R. Harris. 1982. Triumph, a new winter-productive tall fescue variety. Alabama Agric. Exp. Bull. 260.

Howard, M. D., R. B. Muntifering, N. W. Bradley, G. E. Mitchell, Jr., and S. R. Lowry. 1992. Voluntary intake and ingestive behavior of steers grazing Johnstone or endophyte-infected Kentucky-31 tall fescue. J. Anim. Sci. 70: 1227-1237.

Judkins, M. B., D. W. Holcombe, B. W. Hess, L. J. Krysl, M. A. Branine, and J. D. Hess. 1997. Ardacin for steers grazing endophyte-free fescue pasture: effects on live weight gain, forage intake, nitrogen and fiber digestion, ruminal fluid kinetics, ruminal fermentation, and serum hormones and metabolites. J. Anim. Sci. 75: 1100-1111.

Jung, G. A., J. A. Shaffer, and J. R. Everhart. 1994. Fall management effect on Grasslands Matua prairie grass production and sward characteristics. Agron. J. 86: 1032-1039.

Kallenbach, R. L., G. J. Bishop-Hurley, M. D. Massie, G. E. Rottinghaus, and C. P. West. 2003. Herbage mass, nutritive value, and ergovaline concentration of stockpiled tall fescue. Crop Sci. 43: 1001-1005.

Kallenbach, R. L., C. A. Roberts, T. R. Lock, D. H. Keisler, M. R. Ellersieck, and G. E. Rottinghaus. 2006. Performance of steers fed ammoniated straw from tall fescue seed fields. Online. Forage and Grazinglands. doi:10.1094/FG-2006-0113-01-RS.

Kaneko, J., J. W. Harvey, and M. L. Bruss. 1997. Clinical Biochemistry of Domestic Animals. 5th ed. Academic Press, San Diego Calif.

Keren, E. N., and B. E. Olson. 2006. Thermal balance of cattle grazing winter range: Model application. J. Anim. Sci. 84: 1238-1247.

Klopfenstein, T. J., D. J. Jordan, I. Rush, and T. Milton. 1999. Predicting amount of compensatory gain. Nebraska Beef Cattle Report. MP 71-A:: 26-28. Lincoln, NE.

LaCasha, P. A., H. A. Brady, V. G. Allen, C. R. Richardson, and K. R. Pond. 1999. Voluntary intake, digestibility, and subsequent selection of matua bromegrass, coastal bermudagrass and alfalfa hays by yearling horses. J. Anim. Sci. 77: 2766-2773.

Lacefield, G., and J. K. Evans. 1984. Tall fescue in Kentucky. AGR-108, Univ. of KY, Lexington, KY.

Lakritz, J., M. J. Leonard, P. A. Eichen, G. E. Rottinghaus, G. C. Johnson, and D. E. Speirs. 2002. Whole-blood concentrations of glutathione in cattle exposed to heat stress or a combination of heat stress and endophyte-infected tall fescue toxins in controlled environmental conditions. Am. J. Vet. Res. 63: 799-803.

Laredo, M. A., and D. J. Minson. 1973. The voluntary intake, digestibility, and retention time by sheep of leaf and stem fractions of five grasses. Aust. J. Agric. Res. 24: 875-888.

Larson, B. T., E. L. Harmon, E. L. Piper, L. M. Griffis, and L. P. Bush. 1999. Alkaloid binding and activation of D2 dopamine receptors in cell culture. J. Anim. Sci. 77: 942-947.

Latch, G. C. M. 1997. An overview of *Neotyphodium*-grass interactions. In: C. W. Bacon and N. S. Hill (eds.) *Neotyphodium*/Grass Interactions. p 1-4. Plenum Press, New York.

Latch, G. C. M., M. J. Christensen, B. A. Tapper, H. S. Easton, D. E. Hume, and L. R. Fletcher. 2000. Tall fescue endophytes No. U.S. Patent 6111170. Date issued: 29 August.

Lipham, L. B., F. N. Thompson, J. A. Stuedemann, and J. L. Sartin. 1989. Effects of metoclopramide on steers grazing endophyte-infected fescue. J. Anim. Sci. 68: 1245-1253.

Lippke, H. 2002. Estimation of Forage Intake by Ruminants on Pasture. Crop Sci 42: 869-872.

Livak, K. J., and T. D. Schmittgen. 2001. Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2-ΔΔCt Method. Methods 25: 402-408.

Lodge-Ivey, S. L., K. Walker, T. Fleischmann, J. E. True, and A. M. Craig. 2006. Detection of lysergic acid in ruminal fluid, urine, and in endophyte-infected tall

fescue using high-performance liquid chromatography. J. Vet. Diagn. Invest. 18: 369-374.

Lopez-Guerrero, I. 2005. Estimating Forage Mass of Tall Fescue Pastures and Dry Matter Intake and Digestibility of Fescue Forages by Beef Steers, Ph.D. Dissertation, Virginia Tech, Blacksburg, VA.

Lowe, K. F., T. M. Bowdler, N. D. Casey, and R. J. Moss. 1999. Performance of temperate perennial pastures in the Australian subtropics. 1. Yield, persistence and pasuture quality. Aust. J. Exp. Agric. 39: 663-676.

Malinowski, D., H. Zuo, D. Belesky, and G. Alloush. 2004. Evidence for copper binding by extracellular root exudates of tall fescue but not perennial ryegrass infected with *Neotyphodium* spp. endophytes. Plant and Soil 267: 1-12.

Malinowski, D. P., G. A. Alloush, and D. P. Belesky. 1998. Evidence for chemical changes on the root surface of tall fescue in response to infection with the fungal endophyte Neotyphodium coenophialum. Plant Soil 205: 1-12.

Mayes, R. W., and C. S. Lamb. 1984. The possible use of n-alkanes in herbage as indigestible fecal markers. Proc. Nutr. Soc. 43: 39A (abstract).

Mayes, R. W., C. S. Lamb, and P. M. Colgrove. 1986a. Determination of herbage intake of suckling lambs using long-chained n-alkanes as markers. Anim. Prod. Aust. 42: 457.

Mayes, R. W., C. S. Lamb, and P. M. Colgrove. 1986b. The use of dosed and herbage n-alkanes as markers for the determination of herbage intake. J. Agric. Sci. 107: 161-170.

Mayes, R. W., C. S. Lamb, and P. M. Colgrove. 1988. Digestion and metabolism of dosed even-chain adn herbage odd-chain n-alkanes in sheep. Proc. 12th Gen. Meeting Eur. Grasslds Fed.: pp. 159-163.

McCord, J. M., and I. Fridovich. 1969. Superoxide dismutase: an enzymatic function for erythrocuprein. J. Biol. Chem. 244: 6049-6055.

McCracken, B. A., L. J. Krysl, K. K. Park, D. W. Holcombe, and M. B. Judkins. 1993. Steers grazing endophyte-free tall fescue: Seasonal changes in nutrient quality, forage intake, digesta kinetics, ruminal fermentation, and serum hormones and metabolites. J. Anim. Sci. 71: 1588-1595.

McDowell. 1994. Copper and Molybdnum Minerals in Animal and Human Nutrition. p 196-204 Acad. Press, New York.

Merchen, N. R. 1988. Digestion, absorption, and excretion in ruminants. In: D. C. Church (ed.) The Ruminant Animal: Digestive Physiology and Nutrition. p 172-201. Waveland Press, Inc., Long Grove, IL, USA.

Miltimore, J. E., and J. L. Mason. 1971. Copper to molybdenum ratio and molybdenum and copper concentrations in rumianant feeds. Can. J. Anim. Sci. 51: 193-200.

Minson, D. J. 1990. Forage in Ruminant Nutrition. Academic Press, San Diego, CA.

Mizuno, N. S., V. Perman, F. W. Bates, J. H. Sautter, and M. O. Schultze. 1959. Life Span of Thrombocytes and Erythrocytes in Normal and Thrombocytopenic Calves. Blood 14: 708-719.

Montgomery, J. L., V. G. Allen, K. R. Pond, M. F. Miller, D. B. Wester, C. P. Brown, R. Evans, C. P. Bagley, R. L. Ivy, and J. P. Fontenot. 2001. Tasco-Forage: IV.

Influence of a seaweed extract applied to tall fescue pastures on sensory characteristics, shelf-life, and vitamin E status in feedlot-finished steers. J. Anim Sci. 79: 884-894.

Moore, J. E., and L. E. Sollenberger. 1997. Techniques to predict pasture intake. In: J.A. Gomide (ed.) Int. Symp. on Animal Production Under Grazing, Vicosa, Brazil. p 81-96.

Moshtaghi, S. A., and K. M. Wittenberg. 2002. Evaluation of n-alkanes as markers for estimation of dry matter intake and digestibility in steers consuming all-forage or forage-concentrate diets. Can. J. Anim. Sci. 82: 419-425.

Muchovej, R. M., V. G. Allen, D. C. Martens, L. W. Zelazny, and D. R. Notter. 1986. Aluminum, citric acid, nitriloacetic acid, and soil moisture effects on aluminum and iron concentration in ryegrass. Agron. J. 78: 138-145.

Neill, J. C. 1940. The enodphytes of *Lolium* and *Festuca*. N. Zeal. J. Sci. Technol 23: 185A-193A.

Nelson, C. J., and L. E. Moser. 1995. Plant factors affecting forage quality. In: G. C. Fahey, Jr., M. Collins, D. R. Mertens and L. E. Moser (eds.) Forage Quality, Evaluation, and Utilization. p 115-154. Am. Soc. of Agron. Inc; Crop Sci. Soc. Am. Inc.; Soil Sci. Soc. Am. Inc., Madison, WI.

Nihsen, M. E., E. L. Piper, C. P. West, R. J. Crawford, Jr., T. M. Denard, Z. B. Johnson, C. A. Roberts, D. A. Spiers, and C. F. Rosenkrans, Jr. 2004. Growth rate and physiology of steers grazing tall fescue inoculated with novel endophytes. J. Anim Sci. 82: 878-883.

NOAA. 1976. Livestock weather stress. U.S. Dept. of Com., National Oceanic and Atmospheric Admin, National Weather Service Central Regioin. Regional Operations Manual Lett: C-31-76.

NRC. 2000. Nutrient Requirements of Beef Cattle. 7th ed. Natl. Acad Press, Washington, D.C.

Oliver, J. W. 2005. Pathophysiological response to endophyte toxins. In: C. A. Roberts, C. P. West and D. A. Spiers (eds.) Neotyphodium in Cool-Season Grasses. Blackwell Publ., Ames, IA.

Oliver, J. W., L. K. Abney, J. R. Strickland, and R. D. Linnabary. 1993. Vasoconstriction in bovine vasculature induced by the tall fescue alkaloid lysergamide. J. Anim. Sci. 71: 2708-2713.

Oliver, J. W., A. E. Schultze, B. W. Rohrbach, H. A. Fribourg, T. Ingle, and J. C. Waller. 2000. Alterations in hemograms and serum biochemical analytes of steers after prolonged consumption of endophyte-infected tall fescue. J. Anim Sci. 78: 1029-1035.

Oliver, J. W., J. R. Strickland, J. C. Waller, H. A. Fribourg, R. D. Linnabary, and L. K. Abney. 1998. Endophytic fungal toxin effect on adrenergic receptors in lateral saphenous veins (cranial branch) of cattle grazing tall fescue. J. Anim Sci. 76: 2853-2856.

Oro, J., and W. Nooner. 1965. Paraffinic hydrocarbons in pasture plants. Science 147: 870-873.

Owens, F. N., and C. F. Hanson. 1992. External and internal markers for appraising site and extent of digestion in ruminants. J. Dairy Sci. 75: 2605-2617.

Panaccione, D. G., R. D. Johnson, J. Wang, C. A. Young, P. Damrongkool, B. Scott, and C. L. Schardl. 2001. Elimination of ergovaline from a grass-Neotyphodium endophyte symbiosis by genetic modification of the endophyte. Proc. Natl. Acad. Sci. USA 98: 12820-12825.

Panaccione, D. G., and C. L. Schardl. 2003. Molecular genetics of ergot alkaloids. In: J. F. White, Jr., C. W. Bacon, N. L. Hywel-Jones and J. W. Spatafora (eds.) Fungi: Evolutionary Biology, Chemistry, Biocontrol, and Cultural Impacts. p 399-424. Marcel Dekker, New York.

Parish, J. A., M. A. McCann, R. H. Watson, C. S. Hoveland, L. L. Hawkins, N. S. Hill, and J. H. Bouton. 2003a. Use of nonergot alkaloid-producing endophytes for alleviating tall fescue toxicosis in sheep. J. Anim Sci. 81: 1316-1322.

Parish, J. A., M. A. McCann, R. H. Watson, N. N. Paiva, C. S. Hoveland, A. H. Parks, B. L. Upchurch, N. S. Hill, and J. H. Bouton. 2003b. Use of nonergot alkaloid-producing endophytes for alleviating tall fescue toxicosis in stocker cattle. J. Anim Sci. 81: 2856-2868.

Parsons, A. J., J. A. Newman, P. D. Penning, A. Harvey, and R. J. Orr. 1994. Diet preference of sheep: effects of recent diet, physiological state and species abundance. J. Anim. Ecol. 63: 465-478.

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

Paynter, D. J. 1987. The diagnosis of copper insufficiency. In: J. M. Howell and J. M. Gawthorne (eds.) Copper in Animals and Man. Vol. 1. p 101-119. CRC Press, Boca Raton, FL.

Petroski, R. J., and R. G. Powell. 1991. Preperative separation of complex alkaloid mixture by high-speed countercurrent chromatography. In: P. A. Hedin (ed.) Naturally Ocurring Pest Bioregulators. p 436-434. Am. Chem. Soc., Washington, DC.

Piasentier, E., S. Bovolenta, F. Malossini, and P. Susmel. 1995. Comparison of n-alkanes or chromium oxide methods for estimation of herbage intake by sheep. Small Rum. Res. 18: 27-32.

Porter, J. K. 1995. Analysis of endophyte toxins: fescue and other grasses toxic to livestock. J. Anim Sci. 73: 871-880.

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

Powell, R. G., R. J. Petroski, and K. Clay. 1991. Alkaloids of *Stipa robusta* ("sleepy grass") infected with an *Acremonium* endophyte as compared to the alkaloid of tall fescue. In: Proc. Tall Fescue Toxicosis Workshop, SERA-IEG-37, Atlanta, GA. p 37-49.

Puoli, J. R., G. A. Jung, and R. L. Reid. 1991. Effects of nitrogen and sulfur on digestion and nutritive quality of warm-season grass hays for cattle and sheep. J. Anim. Sci. 69: 843-852.

Puschner, B., Y. K. Choi, J. H. Tegzes, and M. C. Thurmond. 2004. Influence of age, sex, and production class on liver zinc concentration in calves. J. Vet. Diagn. Invest. 16: 278-282.

Redmon, L. A., F. T. McCollum, III, G. W. Horn, M. D. Cravey, S. A. Gunter, P. A. Beck, J. M. Mieres, and R. San Julian. 1995. Forage intake by beef steers grazing winter wheat with varied herbage allowances. J. Range Manag. 48: 198-201

Redmond, L. M., D. L. Cross, J. R. Strickland, and S. W. Kennedy. 1994. Efficacy of domperidone and sulpiride as treatment for fescue toxicosis in horses. Am. J. Vet. Res. 55: 722-729.

Roberts, C. A., R. L. Kallenbach, and N. S. Hill. 2002. Harvest and storage method affects ergot alkaloid concentration in tall fescue. Online. Crop Mang. doi:10.1094/CM-2002-0917-01-BR.

Rottinghaus, G. E., G. B. Garner, C. N. Cornell, and J. L. Ellis. 1991. HPLC method for quantitating ergovaline in endophyte infested tall fescue: Seasonal variation of ergovaline levels in stems with leaf sheaths, leaf blades, and seed heads. J. Agric. Food Chem. 39: 112-115.

Rotz, J. D. 2006. Comparison of techniques for estimating pasture herbage mass and productive ground cover for Lakota prairie grass, Kentucky 31 endophyte free tall fescue, Kentucky 31 endophyte infected tall fescue and Quantum 542 tall fescue grazed by stocker cattle, M.S. Thesis, Virginia Tech, Blacksburg.

Roylance, J. T., N. S. Hill, and C. S. Agee. 1994. Ergovaline and peramine production in endophyte-infected tall fescue: independent regualtion and effects of plant and endphytic genotype. J.Chem. Ecol. 20: 2171-2183.

Rumball, W., and J. E. Miller. 2003. 'Grasslands Lakota' prairie grass (*Bromus catharticus* Vahl.). N.Z. Exp. Agric. 46: 61-63.

Saker, K. E., V. G. Allen, J. P. Fontenot, C. P. Bagley, R. L. Ivy, R. R. Evans, and D. B. Wester. 2001. Tasco-Forage: II. Monocyte immune cell response and performance of beef steers grazing tall fescue treated with a seaweed extract. J. Anim Sci. 79: 1022-1031.

Saker, K. E., V. G. Allen, J. Kalnitsky, C. D. Thatcher, W. S. Swecker, Jr., and J. P. Fontenot. 1998. Monocyte immune cell response and copper status in beef steers that grazed endophyte-infected tall fescue. J. Anim Sci. 76: 2694-2700.

Salminen, S. O., P. S. Grewal, and M. F. Quigley. 2003. Does mowing height influence alkaloid production in endophytic tall fescue and perennial rygrass? J. Chem. Ecol. 29: 1319-1328.

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

Scaglia, G., H. T. Boland, I. Lopez-Guerrero, R. K. Shanklin, and J. P. Fontenot. 2005. Use of alkanes to estimate dry matter intake of beef steers grazing high quality pastures. Proc. XX International. Grassland Congress: 250.

Schardl, C. L., J. D. Blankenship, C. Machado, and M. J. Spiering. 2002. Alkaloid-making Fungal Symbionts. In: L. Taiz and E. Zeiger (eds.) Plant Physiology Online. Sinauer Assoc., Sunderland, MA

Schardl, C. L., A. Leuchtmann, and M. J. Spiering. 2004. Symbioses of grasses with seedborne fungal endophytes. Annual Review of Plant Biology 55: 315-340.

Seman, D. H., J. A. Stuedemann, and J. E. Anderson. 1997. Spectral analysis of bovine grazing behavior on *Neotyphodium coenophialum* infested tall fescue. Appl. Anim. Behav. Sci. 54: 73-87.

Settivari, R. S., S. Bhusari, T. Evans, P. A. Eichen, L. B. Hearne, E. Antoniou, and D. E. Spiers. 2006. Genomic analysis of the impact of fescue toxicosis on hepatic function. J. Anim. Sci. 84: 1279-.

Siegel, M. R., G. C. M. Latch, and M. C. Johnson. 1987. Fungal endophytes of grasses. Annu. Rev. Phytopathol. 25: 293-315.

Sleper, D. A. 1979. Plant breeding, selection, and species in relation to grass tetany. In: V. V. Rendig and D. L. Grunes (eds.) Grass Tetany. Special Publication. No. 35.

Sleper, D. A., H. F. Mayland, R. J. Crawford, Jr., G. E. Shewmaker, and M. D. Massie. 2002. Registration of HiMag tall fescue germplasm. Crop Sci. 42: 318.

Sollenberger, L. E., and D. J. R. Cherney. 1995. Evaluating forage production and quality. In: R. F. Barnes, D. A. Miller and C. J. Nelson (eds.) Forages. Vol II: The Science of Grassland Agriculture. p 97-110. Iowa State University Press, Ames, IA, USA.

Standish, J. F., C. B. Ammerman, C. F. Simpson, F. C. Neal, and A. Z. Palmer. 1969. Inlfuence of graded levels of dietary iron, as ferrous sulfate, on performance and tissue mineral composition of steers. J. Anim. Sci. 29: 496-503.

Stewart, R. L., Jr. , L. E. Sollenberger, J. C. B. Dubeux, Jr., J. M. B. Vendramini, S. M. Interrante, and Y. C. Newman. 2006. Herbage and animal responses to management intensity of continuously stocked bahiagrass pastures. Agron. J. In Press.

Strickland, J. R., D. L. Cross, G. P. Birrenkott, and H. W. Grimes. 1994. Effect of ergovaline, loline, and dopamine antagonists on rat pituitary cell prolactin release in vitro. Am. J. Vet. Res. 55: 716-721.

Strickland, J. R., D. L. Cross, T. C. Jenkins, R. J. Petroski, and J. B. Powell. 1992. The effect of alkaloids and seed extracts of endophyte-infected tall fescue on prolactin secretion in an in vitro rat pituitary perfusion system. J. Anim. Sci. 70: 2779-2786.

Stuedemann, J. A., N. S. Hill, F. N. Thompson, R. A. Fayrer-Hosken, W. P. Hay, D. L. Dawe, D. H. Seman, and S. A. Martin. 1998. Urinary and biliary excretion of ergot alkaloids from steers that grazed endophyte-infected tall fescue. J. Anim Sci. 76: 2146-2154.

Stuedemann, J. A., and C. S. Hoveland. 1988. Fescue endophyte history and impact on animal agriculture. J. Prod. Agric. 1: 39-44.

Stuedemann, J. A., and F. N. Thompson. 1993. Management strategies and potential opportunities to reduce the effects of endophyte-infected tall fescue on animal performance. In: D. E. Hume (ed.) Int. Symp. on *Acremonium*/Grass Interactions. p 103-114. AgRes. Grassl., Palmerston North, New Zealand.

Suttle, N. F., and D. G. Jones. 1987. Heinz body anaemia in lambs with deficiencies of copper or selenium. Br. J. Nutr. 58: 539-548.

Suttle, N. F., and C. H. McMurray. 1983. Use of erythrocyte coper:zinc superoxide dismutase activity and hair or fleece concentrations in the diagnosis of hypocuprosis. Res. Vet. Sci. 35: 47-52.

Thiele, D. J. 1992. Metal-regulated transcription in eukaryotes. Nucleic Acids Res. 20: 1183-1191.

Thompson, F. N., J. A. Stuedemann, and N. S. Hill. 2001. Anti-quality factors associated with alkaloids in eastern temperate pastures. J. Range Manage. 54: 474-489.

Thompson, R. W., H. A. Fribourg, J. C. Waller, W. L. Sanders, J. H. Reynolds, J. M. Phillips, S. P. Schmidt, R. J. Crawford, Jr., V. G. Allen, D. B. Faulkner, and a. et. 1993. Combined analysis of tall fescue steer grazing studies in the Eastern United States. J. Anim. Sci. 71: 1940-1946.

Tsai, H. F., H. Wang, J. C. Gebler, C. D. Poulter, and C. L. Schardl. 1995. The Claviceps purpurea Gene Encoding Dimethylallyltryptophan Synthase, the Committed Step for Ergot Alkaloid Biosynthesis. Biochemical and Biophysical Research Communications 216: 119-125.

Tudzynski, P., T. Correia, and U. Keller. 2001. Biotechnology and genetics of ergot alkaloids. Appl. Microbiol. Biotechnol. 57: 593-605.

Uauy, R., M. Olivares, and M. Gonzalez. 1998. Essentiality of copper in humans. Am. J. Clin. Nutr. 67(suppl): 952S-959S.

Unal, Y., and P. C. Garnsworthy. 1999. Estimation of intake and digestibility of forage-based diets in group-fed dairy cows using alkanes as markers. J. Agric. Sci. 133: 419-425.

Underwood, E. J., and N. F. Shuttle. 1999. The Mineral Nutrition of Livestock. 3rd ed. CAB Int., New York, NY.

VAES. 2006. College Farm Operations, College of Agriculture and Life Sciences, weather data. Available: http://www.vaes.vt.edu/colleges/kentland/weather/ Accessed January 28, 2006.

Vallentine, J. F. 2001. Grazing Management. 2nd ed. Academic Press, New York.

Vazquez, O. P., and T. R. Smith. 2000. Factors Affecting Pasture Intake and Total Dry Matter Intake in Grazing Dairy Cows. J. Dairy Sci. 83: 2301-2309.

VDACS. 2005. Virginia Department of Agriculture adn Consumer Services. Comodity Report. Accessed: October 29, 2005: available: http://www.vdacs.virginia.gov/.

Vulich, S. A., E. G. O'Riordan, and J. P. Hanrahan. 1991. Use of n-alkanes for the estimation of herbage intake in sheep: Accuracy and precision of estimates. J. Agric. Sci. 116: 319-323.

Waller, J. C., and H. A. Fribourg. 2002. Performance of steers grazing pastures of endophyte-infested and endophyte-free tall fescue with and without clover. Univ. of Tenn. Dept. Anim. Sci. Annul. Report. animalscience.ag.utk.edu/annualreports.htm.

Waller, J. C., H. A. Fribourg, R. J. Carlisle, G. C. M. Latch, L. R. Fletcher, R. J. M. Hay, H. S. Eason, and B. A. Tapper. 2001. Tall fescues with nontoxic endophytes in Tennessee, Annual Dept. Reports, Univ. of Tenn., Knoxville, TN.

Waller, J. C., H. A. Fribourg, A. E. Schultze, B. W. Rohrbach, and J. W. Oliver. 2002. Effect of grazing endophyte-infested tall fescue on blood components and performance of beef steers, Univ. of Tenn., Knoxville, TN.

Ward, J. D., and J. W. Spears. 1997. Long-term effects of consumption of low-copper diets with or without supplemental molybdenum on copper status, performance, and carcass characteristics of cattle. J. Anim. Sci. 75: 3057-3065.

Ward, J. D., J. W. Spears, and E. B. Kegley. 1993. Effect of copper level and source (copper lysine vs copper sulfate) on copper status, performance, and immune response in growing steers fed diets with or without supplemental molybdenum and sulfur. J. Anim. Sci. 71: 2748-2755.

Weir, W. C. 1959. Selective grazing by sheep as shown bya comparison of the chemical composition of range and pasture forage obtained by hand clipping and that collected by esophageal-fistulated sheep. J. Anim. Sci. 18: 641-649.

West, E. C., and J. R. Prohaska. 2004. Cu,Zn-Superoxide Dismutase Is Lower and Copper Chaperone CCS Is Higher in Erythrocytes of Copper-Deficient Rats and Mice. Exp. Bio.Med. 229: 756-764.

Westendorf, M. L., G. E. Mitchell, Jr., R. E. Tucker, L. P. Bush, R. J. Petroski, and R. G. Powell. 1993. In vitro and in vivo ruminal and physiological responses to endophyte-Infected tall fescue. J. Dairy Sci. 76: 555-563.

White, J. F., Jr. 1994. Systematics of the Graminicolous Clavicipitaceae: Applications of morphological and molecular approaches. In: C. W. Bacon and N. S. Hill (eds.) Neotyphodium/Grass Interactions. Plenum Press, New York.

Wikse, S. E., D. Herd, R. Field, and P. Holland. 1992. Diagnosis of copper deficiency in cattle. J. Am. Vet. Med. Assoc. 200: 1625-1629.

Wilkinson, H. H., M. R. Siegel, J. D. Blankenship, A. C. Mallory, L. P. Bush, and C. L. Schardl. 2000. Contribution of fungal loline alkaloids to protection from aphids in a grass-endophyte mutualism. Mol. Plant Microbe Interact. 13: 1027-1033.

VITA

Robert Lawton Stewart, Jr, the son of Robert and Martha Stewart, was born June 29, 1979 in Columbus, Mississippi. Lawton grew up in Tifton, Georgia and graduated from Tift County High School in 1997. After high school, he attended the University of Georgia and received his B.S. in Animal Science in 2001. Lawton then pursued a Master of Science in Agronomy at the University of Florida, studying Forage Management, and received his degree in 2003. He then continued his graduate career, pursuing a Doctor of Philosophy degree at the Virginia Polytechnic Institute and State University as a John Lee Pratt fellow in Nutrition. At Virginia Tech, he studied Ruminant Nutrition under the guidance of Guillermo Scaglia. Lawton is married to his wife, Beth, and has one son, Thomas.