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EFFECTS OF ENDOPHYTE INFECTED FESCUE ALKALOID INGESTION ON ENERGY METABOLISM, NITROGEN BALANCE, IN SITU FEED DEGRADATION, AND RUMINAL PASSAGE RATES

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EFFECTS OF ENDOPHYTE INFECTED FESCUE ALKALOID INGESTION ON
ENERGY METABOLISM, NITROGEN BALANCE, IN SITU FEED
DEGRADATION, AND RUMINAL PASSAGE RATES

DISSertation

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the
College of Agriculture at the University of Kentucky

By
Anne Fleming Koontz
Lexington, Kentucky

Director: Dr. David Harmon, Professor of Animal Science
Lexington, Kentucky

2013

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ABSTRACT OF DISSERTATION

EFFECTS OF ENDOPHYTE INFECTED FESCUE ALKALOID INGESTION ON ENERGY METABOLISM, NITROGEN BALANCE, *IN SITU* FEED DEGRADATION, AND RUMINAL PASSAGE RATES

The decrease in productivity caused by fescue toxicosis has been estimated to cost the United States livestock industry more than $1 billion per year due to reduced growth and diminished reproductive efficiency. This goal of the research presented in this dissertation is to enhance the knowledge base concerning the underlying physiological changes that occur during fescue toxicosis that lead to reduced intake and weight gain in cattle.

As one of the factors associated with fescue toxicosis is a reduction in feed intake, achieving a consistent and adequate intake of toxins can be a complication. Results from experiment 1 demonstrate that ruminal dosing of ground seed and a seed extract are able to mimic the classic symptoms of fescue toxicosis in cattle. This model whereby seed or extract is directly dosed into the rumen eliminates the possibility of reduced alkaloid intake due to refusal of feed by the animal. This model allows for more precise and repeatable dosing of alkaloids in fescue research.

Experiment 2 results indicate that ingestion of endophyte-infected tall fescue leads to decreased fasting heat production in cattle. This is indicative of a reduction in maintenance energy requirements and may be related to a decrease in liver size or other metabolic activity in animals grazing endophyte-infected pastures. In addition, a reduction in basal metabolic rate may cause the compensatory gain often observed in cattle entering the feedlot after grazing endophyte-infected pastures.
Data from experiment 3 provides evidence that whole body nitrogen and energy metabolism are not altered by fescue alkaloid ingestion. Experiment 3 also addresses the rate of feed degradation and ruminal passage rates in cattle ingesting endophyte infected fescue. While ruminal VFA profile is altered, this is likely due to reduced absorption, not increased production. The data from this experiment indicate that the reduction in weight gain and productivity seen during fescue toxicosis is primarily a function of reduced intake and not secondary effects of alkaloid ingestion.

KEYWORDS: fasting heat production, fescue toxicosis, nutrient metabolism, passage rate, ruminant

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February 14, 2013
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This dissertation is dedicated to those who have supported and encouraged me throughout my life. My parents, Bill and Sherry Beckemeyer, who gave me the desire to never stop learning and taught me to follow my passions. My brother Curtis and Sister Catherine, who reminded me to take a break now and then to see where my research and dreams met the real world. Friends to numerous to name, who kept me laughing and sane through it all. But, most of all, my husband Paul, who married into this wild ride and helped me with every step of the research, long nights, and weekends. Without him I might still be working. I am grateful for your love and support.
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FREQUENTLY USED ABBREVIATIONS

5-HT: 5-hydroxytryptamine (serotonin) receptor
ADG: average daily gain
BW: body weight
CH₄: methane
CO₂: carbon dioxide
CP: crude protein
d: day
D2: dopamine receptor 2
DE: digestible energy
DM: dry matter
DMI: dry matter intake
E-: endophyte uninfected
E+: endophyte infected
FHP: fasting heat production
GIT: gastrointestinal tract
h: hour
hd: head (i.e. animal)
LCFA: long chain fatty acid
ME: Metabolizable energy
min: minute
N: nitrogen
NEm: net energy for maintenance
O₂: oxygen
PRL: prolactin
RE: retained energy
RQ: respiratory quotient (CO₂ produced / O₂ consumed)
VFA: volatile fatty acid
CHAPTER 1: Introduction

Tall fescue (*Lolium arundinaceum*) is grown on more than 15 million hectares land in the United States (Thompson et al., 1993) and more than half of these fields are infected with the fungal endophyte *Neotyphodium coenophialum* (Jones et al., 2004a). While the symbiotic relationship with this endophyte provides positive aspects such as drought and heat tolerance to the plant (Settivari et al., 2006), the alkaloids produced by the endophyte cause health and production problems in animals grazing the infected fescue resulting in negative economic effects for producers (Hoveland, 2007). The decrease in productivity caused by fescue toxicosis has been estimated to cost United States beef producers more than $600 million per year due to reduced growth, diminished reproductive efficiency, and market discrimination due to unthrifty appearance (Paterson et al., 1995). A more recent report estimates the losses at more than $1 billion for the entire livestock industry (Strickland et al., 2011).

The clinical signs of fescue toxicosis include reduced feed intake and weight gain, decreased milk production, reduced reproductive efficiency, tissue necrosis, and a rough hair coat (Wagner, 2008). Physiological symptoms including increased respiration rate (Browning and Leite-Browning, 1997; Al-Haidary et al., 2001), elevated core temperature (Hannah et al., 1990; Rhodes et al., 1991; Aldrich et al., 1993b), and a reduction in serum prolactin (Oliver, 1997) can be used to diagnose less severe cases of fescue toxicosis. These symptoms are caused by the action of ergot alkaloids on a variety of receptors and tissues within the body, including α1- and α2-adrenergic, serotonin-2 (5-HT), and dopaminergic (D2) receptors.

The most easily measured effect of fescue toxicosis is a significant decrease (up to 50%) in intake (Bond et al., 1984; Stuedemann and Hoveland, 1988). Considering the variety of known physiological effects of ergot alkaloids, it is unlikely that this reduction in intake is the sole cause for the negligible weight gain seen in these animals. Changes in organ mass, gene expression, and stress can affect energy metabolism, altering nutrient availability and use. Each of these can be affected by both reduced nutrient intake and alkaloid
consumption. Several studies indicate that consumption of ergot alkaloids and reduced energy intake may interact to alter energy metabolism in rats consuming endophyte-infected tall fescue (Zhang et al., 2002; Spiers et al., 2005a). However, minimal research has been conducted to separate the effects of reduced energy intake and alkaloid consumption.

As endophyte toxins are concentrated in tall fescue seed, research examining the effects of endophyte infected tall fescue on animal performance often utilizes ground seed added to a basal diet to induce the symptoms of fescue toxicosis. As one of the factors associated with fescue toxicosis is a reduction in feed intake (Rhodes et al., 1991; Aldrich et al., 1993a), achieving a consistent intake of alkaloids is difficult. A more precise method of disease induction would be to ruminally dose the animals with ground seed or an extract containing the alkaloids from endophyte infected tall fescue. However, little research has been done to determine the feasibility of ruminal dosing or the bioactivity of such extracts.

It is well known that level of intake affects ruminal passage rate and in turn, passage rate affects digestion of feeds (Church, 1988; NRC, 1996). Gastro-intestinal tract (GIT) motility can also significantly affect digestibility of feed and passage rate (Church, 1988). Several of the receptor types acted on by ergot alkaloids have been implicated in control of GIT motility (Talley, 1992; Oliver et al., 1993; van Miert et al., 1994; Schoning et al., 2001). Previous research examining the digestibility of tall fescue by comparing infected and uninfected forages or seeds has provided mixed results (Goetsch et al., 1987; Hannah et al., 1990; Humphry et al., 2002). Additionally, there is a lack of research examining the digestibility of other feedstuffs in animals consuming endophyte infected fescue; and of digestibility of a common feedstuff in animals consuming endophyte infected and uninfected diets. As a common method for ameliorating the effects of infected tall fescue is supplementation with alternate feeds or co-seeding pastures with a legume (Smith et al., 1975), it is crucial to understand the effect ergot alkaloid ingestion may have on digestion and utilization of such feeds.
The purpose of this dissertation is to evaluate a novel method of inducing fescue toxicosis and utilize this method to separate the effects of ergot alkaloid ingestion and level of feed intake when evaluating alterations in energy and nitrogen balance, as well as digestive characteristics. Chapter 2 provides a review of the literature concerning the history of fescue toxicosis research, as well as the current understanding of the underlying physiological mechanisms. The preliminary study to evaluate a novel method of fescue toxicosis induction is reported in Chapter 3, indicating that a 5-7 day ruminal dosing protocol is a repeatable method of inducing fescue toxicosis symptoms in cattle. Chapter 4 discusses research examining the alteration of fasting heat production and basal metabolism during fescue toxicosis. Chapter 5 examines the effect of fescue toxicosis on whole body nitrogen and energy balance. Effects of alkaloid ingestion on feed degradation of a common feedstuff and ruminal kinetics is also presented in Chapter 5. This dissertation research enhances the knowledge base concerning the underlying physiological changes that occur during fescue toxicosis that lead to reduced intake and weight gain in cattle.
2.1) History

Tall Fescue (*Lolium arundinaceum*), a native grass of Europe, was first imported to the United States in the 1800s (Kennedy, 1900). By the end of the 19th century, tall fescue was described as “an exceedingly valuable grass for mowing or pasture” due to its “superior growth, height, competitive ability, and drought tolerance” (Hoveland, 2007). However, tall fescue was not widely planted until the 1940s and ‘50s when a variety developed by the University of Kentucky (KY-31) was seeded on over 35 million acres in the Southeastern United States (Lacefield, 2006). By 1973, tall fescue had become the predominant cool-season grass in the US, particularly in the South-East (Buckner and Bush, 1979). It gained popularity because it was highly adaptable, grew well in drought conditions and on poor soils, and due to its deep root system could be used to stabilize heavily eroded areas (Buckner and Bush, 1979). Laboratory analysis of tall fescue indicates that it is a high quality forage and should allow for good animal performance (Table 2.1). However, despite this, as early as the 1950s tall fescue began to gain a reputation for causing health and production problems in grazing livestock (Hoveland, 2007).

These problems were categorized into three syndromes: fescue foot, fat necrosis, and summer slump. Fescue foot is associated with soreness in the hind-limbs, hyperemia and swelling of the coronary band, dry gangrene on ear tips and tails, and in severe cases, sloughing of the hoof and tail-switch (Hemken et al., 1984; Wagner, 2008). These symptoms were first described in New Zealand by Cunningham (1949) and are most commonly seen in the fall and winter. Lipomatosis, more commonly called fat necrosis occurs most often in areas with high levels of nitrogen fertilization. Animals grazing these pastures accumulate hard fat along the gastrointestinal tract (GIT), resulting in digestion problems and dystocia (Bush et al., 1979). Summer slump, also simply called fescue toxicosis, is characterized by retention of the winter hair coat, increased respiration rate and body temperature, reduced heat tolerance, poor weight gain, and reduced feed intake (Bush et al., 1979; Hemken et al., 1984; Wagner, 2008).
These syndromes as well as known causative mechanisms are discussed in detail below.

2.2) Economic Cost

Tall fescue is grown on more than 15 million hectares of land in the United States and more than half of these acres are infected with the fungal endophyte *Neotyphodium coenophialum* (Thompson et al., 1993). In the United States an estimated 8.5 million beef cattle graze tall fescue (Hoveland, 1993). The decrease in productivity caused by fescue toxicosis has been estimated to cost United States beef producers more than $600 million per year (Paterson et al., 1995). Reproductive issues result in an estimated $354 million loss due to reduced calf numbers, while reduced weaning weights are estimated to cost $255 million (Hoveland, 1993). When combined with equine and other livestock species, the revenue loss is upwards of $1 billion each year (Strickland et al., 2011).

2.3) Endophyte

2.3.1) Discovery

During the 1970s researchers found a correlation between a number of fungal endophytes and tall fescue plants in Newton Co., Georgia (Bacon, 1995). J.D. Robbins visited a farm in 1973 and observed signs of fescue toxicosis in cattle in one pasture, but not those in the adjacent field (Robbins, 1983), though both were pastures of tall fescue. Microscopic examination of samples from these pastures showed higher levels of the fungal endophyte *Epichloë typhina* (renamed *Acremonium coenophialum* and later *Neotyphodium coenophialum*) in the fields with symptomatic cattle (Robbins, 1983; Hoveland, 2007). This finding was not widely accepted as the cause of fescue toxicosis until it was substantiated by Hoveland et al. (1983) who found that animals grazing the highly infected (94%) pastures gained 0.50 kg/d while those grazing the lowly infected (5%) fescue pastures had an average daily gain (ADG) of 0.83 kg/d.
Unfortunately, the endophyte that causes problems in grazing livestock is also that which gives tall fescue its hardiness.

2.3.2) Life Cycle and Growth

\textit{Neotyphodium} fungi are asexual derivatives of \textit{Epichloë} species (Schardl et al., 2004). The entire lifecycle of the endophytic fungi takes place inside the tall fescue plant, with no external indications of infection (Bacon et al., 1986). Within the plant, the endophyte grows as elongate, sparsely branched hyphae in intercellular spaces (Clay, 1990). The hyphae are approximately 1-2μm thick, and found in all plant structures except the roots and anthers (Schardl et al., 2004). Growth appears to be synchronized to that of the plant, with rapid growth along the longitudinal axis in expanding leaves that stops when leaf elongation is complete (Bacon et al., 1986; Schardl et al., 2004). Propagation of the endophyte occurs by vertical transmission, with the endophyte infecting the seeds of the parent plant as they develop (Schardl et al., 2004).

Early fields found to have low levels of endophyte infection were often found to result from different planting dates or seed lots (Bacon, 1995). Long term storage, high temperatures, or elevated humidity levels can cause the endophyte in the seed to be inviable (Welty et al., 1987; Hill and Roach, 2009), resulting in low or uninfected fields.

2.3.3) Distribution

Endophyte infected tall fescue has been widely planted due to its hardiness, high carrying capacity, improved soil nitrogen (N) use, drought tolerance, and insect resistance (Richardson et al., 1990). Fescue is predominantly used for grazing animals in the Southeastern US, and in the Pacific Northwest (USDA-NRCS, 2009). Shelby and Dalrymple (1987) examined more than 2400 tall fescue tiller and seed samples from across the United States for endophyte infection. Overall, 58.47\% of plants were infected with \textit{N. coenophialum}, as were 38.41\% of the seeds. It is estimated that more than 90\% of tall fescue pastures in the United States are infected with \textit{N. coenophialum}
argile (Siegel et al., 1985; Glenn et al., 1996). While in Kentucky, more that 85% of tall fescue pastures are endophyte infected (Lacefield et al., 2003).

2.4) Ergot Alkaloids

After *Neotyphodium coenophialum* was identified as the important endophytic fungus affecting tall fescue, researchers focused on compounds produced by this endophyte and found that the it had the ability to produce a variety of ergot alkaloids (Bacon, 1995). A mixture of ergopeptides, ergolines, and pyrrolizidine alkaloids, as well as clavines are produced by *N. coenophialum* in tall fescue (Schnitzius et al., 2001). Several of these alkaloids have been detected in the blood of cattle grazing infected pastures (Oliver and Fletcher, 2005). Four classes of ergot alkaloids (Figure 2.1) account for the majority of the alkaloids found in tall fescue; peptines, clavines, lolines, and lysergic acid and its amides (Lyons et al., 1986; Porter, 1995), it has been postulated that this diversity of substances may account for the variety of symptoms of fescue toxicosis.

Ergopeptines have received the most attention as they constitute as much as 50% of the total alkaloid concentration (Lyons et al., 1986). The ergopeptine ergovaline is believed to be the primary causative agent of fescue toxicosis (Lane et al., 1997). Ergovaline accounts for 84 to 97 percent of the total ergopeptine alkaloid concentration, with concentrations as high as 14 mg/kg in sheaths and 1.5 mg/kg in blades of tall fescue plants (Lyons et al., 1986). Welty et al. (1994) compared ergovaline levels in the seed and straw from 30 KY-31 fescue plants over a two year period. They found that ergovaline levels can vary widely from plant to plant (0-1268ng/g in straw and 2-8659ng/g in seed). Average ergovaline levels also varied with year in fescue straw (292ng/g vs 191ng/g), but not in seed (2509ng/g vs 2295ng/g). Similarly, Lyons et al., (1986) found that alkaloid concentrations could vary by more than 10-fold in differing plant parts despite similar levels of fungal infection. As ergovaline levels are highest in the seeds, and cattle have been reported to preferentially eat seed heads (Bransby et al., 1988; Aiken, 2012), some research has examined seed-head removal from tall
fescue in relation to animal performance. This research showed no improvements in weight gain for steers grazing seed-head clipped pastures (Coffey et al., 1994), though increasing stocking rates to reduce seed head emergence did increase gains (Bransby et al., 1988). This led to the conclusion that the alkaloid amount in other portions of the tall fescue plant is sufficient to induce toxicosis. However, more recent work has shown that chemical seed head suppression can result in a 64% increase in average daily gain (Goff, 2012). Though Goff (2012) also reported higher crude protein (CP) for the treated pastures which may account for the increase in ADG.

Loline alkaloids are present in endophyte infected tall fescue as dizophenanthrenes (perloline and perlolidine) and pyrrolizidines (N-acetylloline and N-formlyllooline), with the pyrrolizidines being quantitatively more abundant (Jackson et al., 1984b; Porter, 1995). Total loline concentrations of 3263μg/g in tall fescue seed and 1723μg/g in forage have been reported (Yates et al., 1990). However, as with ergopeptines, these values can vary with season, N fertilization, and other factors including water and temperature stress (Belesky et al., 1987; Bush et al., 1993). Lolines have been shown to be insecticidal and when produced in the tall fescue plant, result in insect resistance (Bush et al., 1993; Dahlman et al., 1997; Young et al., 2010). This alleopathic characteristic of lolines leads them to be considered by many as beneficial alkaloids as they have not been shown to be directly associated with the negative aspects of fescue toxicosis in livestock as ergopeptines and lysergic acid have (Powell and Petroski, 1993; Fletcher, 2010). Some experiments utilizing tall fescue hay with varying levels of loline alkaloids have shown depressed feed intake, and elevated rectal temperatures with high loline levels (Jackson et al., 1984b). However, the studies did not compare other alkaloid levels in the tall fescue, therefore it is unknown if these results are caused by differences in loline or total alkaloid concentrations, and should not be considered to show negative effects of loline consumption.

Lysergic Acid and its amides (i.e. ergine and ergonovine) can be present in concentrations similar to that of ergovaline (Porter, 1995). Ergine has been
found at up to 3336.6ng/g DM in seed and 343.4ng/g DM in plant tissue (Shelby et al., 1997). As most of the alkaloids found in tall fescue are derivatives of lysergic acid, it is possible that this compound is primarily present as a precursor (Bush et al., 1997). As with other alkaloids, lysergic acid amide levels have been shown to vary with management practices and environmental factors. Concentrations of ergonovine showed a quadratic increase with elevated environmental temperatures (Salminen et al., 2005). Ergine and ergonovine have been strongly associated with drunken horse grass (Achnatherum inebians) and sleepygrass (Anchnatherum robustum), which when consumed by equids cause stupor and aversion to grazing (Miles et al., 1996; Schardl et al., 2004). This would indicate that these compounds may be at least partially responsible for reduced intake of endophyte infected fescue by cattle. Lysergic acid and its amides have been shown to biologically active (as discussed below), however, they are not generally as potent as ergopeptines (Oliver et al., 1993; Klotz et al., 2006).

Clavines such as chanoclavine, agroclavine, and penniclavine are biosynthetic precursors to lysergic acid amides and ergopeptines (Floss, 1976; Garner et al., 1993). Therefore, as with lysergic acid, they may be present primarily due to this role, with limited physiological effects in the animal (Bacon, 1995). While most clavines are considered to physiologically inactive in the animal, some clavines have been shown to have insecticidal affects similar to lolines (Clay and Cheplick, 1989). In addition, research with rabbits given choices of ryegrass with varying levels on individual alkaloids, indicated that clavines may reduce the appeal or palatability of the grass, but not the appetite of the animal (Panaccione et al., 2006). Which would indicate that clavines may play a role in reducing intake of cattle grazing endophyte infected tall fescue pastures.

2.4.1) Biosynthesis

The biosynthesis of ergot alkaloids has been studied for several decades. A majority of the research related to ergot alkaloid synthesis has been done in
Claviceps purpurea, the main causative agent of rye ergotism (Schardl and Panaccione, 2005). The precursors to all ergot alkaloids are L-tryptophan and dimethylallyl-pyrophosphate (DMAPP), a melvonic acid derivative (Bush et al., 1997). These are converted to dimethylallyl-tryptophan (DMAT) by DMAT synthase, in what is considered to be the rate-limiting step of this pathway (Bush et al., 1997). DMAT is subsequently converted to chanoclavine-I. This occurs through a series of steps involving α-N-methylation and oxidation to a diene which is epoxized, resulting in cyclization of the C-ring and release of the α-carboxyl group (Schardl and Panaccione, 2005). Cyclization of the D-ring is catalyzed by chanoclavine-I cyclase to produce agroclavine, which is then transformed to lysergic acid via a series of oxidation steps (Schardl and Panaccione, 2005). Derivatives of lysergic acid are the major contributors to the toxic properties of grasses such as tall fescue and rye (Bush et al., 1997). These derivatives are produced by condensation with three amino acids. It is the specific amino acids condensed that determine the ergopeptine produced (Schardl and Panaccione, 2005). Ergovaline, the most studied alkaloid has alanine, valine, and proline at positions I, II, and III respectively (Schardl and Panaccione, 2005).

2.4.2) Extraction and Detection Methods

In order to better study the effects of alkaloids, significant quantities were needed for laboratory study. In 1979, Porter et al. found that ergopeptine alkaloids could be produced from endophytic fungus in broth cultures. Bacon (1988) developed a procedure to grow Neotyphodium coenophialum in a modified culture medium and extract the ergot alkaloids using CHCl₃ and tartaric acid. In the 1990s methods were refined to use freeze dried, ground, and pelleted samples via extraction with chloroform and sodium hydroxide and silica gel cleanup columns (Rottinghaus et al., 1991; Rottinghaus et al., 1993). However, these protocols have a number of drawbacks. They require the use of a silinizing agent to clean all glassware, large quantities of solvent for sample extraction, and scraping of silica gel thin layer chromatography plates to make
the cleanup columns (Yates et al., 1985; 1991; Rottinghaus et al., 1993; Craig et al., 1994). These factors make extraction expensive and time consuming. Craig et al. (1994) developed a new method of extraction that negated many of these issues. Treating glassware with a deactivating agent was found to be unnecessary; a longer extraction time reduced the amount of solvent used in the process; and sample cleanup was simplified by using commercially available surface-treated silica gel SPE columns (Craig et al., 1994). However, use of these methods is expensive and time consuming to produce alkaloid quantities sufficient for in vivo experiments.

Beginning in the 1980s a number of methodologies were developed to determine the alkaloid content in tall fescue extracts. Bacon (1988) utilized chromatography while Yates (1985) developed a method for tandem mass spectrometry. HPLC quickly became the standard method for determining ergovaline content in fescue plant tissue (Schnitzius et al., 2001). This method typically uses ergotamine as an internal standard, as it is an ergopeptine not typically produced by plant endophytes in significant quantities (Yates et al., 1985; Rottinghaus et al., 1991). However, this HPLC procedure is specific for ergovaline, and does not fully quantify the simpler alkaloids (Schnitzius et al., 2001; Spiering et al., 2002). Hill and Agee (1994) developed a method to detect total ergot alkaloid concentrations via ELISA. However, this method was criticized for having variable specificity for individual alkaloids. Further testing found that ergot alkaloids with larger groups did not bind strongly to the monoclonal antibody (15F3.E5) used in this methodology, thus binding efficiency was reduced to 50% for lysergic acid at $10^{-7}$mol/L, and at $10^{-8}$mol/L for ergonovine and ergonovine maleate (Schnitzius et al., 2001). More recent research has worked to develop an HPLC assay to detect and quantify the smaller ergot alkaloids, primarily lysergic acid (Lodge-Ivey et al., 2006). This technique has a recovery of 80% or better in ruminal fluid, urine, and feces (Lodge-Ivey et al., 2006). Combining HPLC with fluorometric detection has also been shown to be an effective method to analyze ergovaline in plasma and milk (Jaussaud et al., 1998; Durix et al., 1999).
2.5) Alkaloid Pharmokinetics

2.5.1) Absorption

2.5.1.1) Monogastrics

The medical field has historically found numerous uses for ergot alkaloids, including relief from migraine headaches, deep vein thrombosis, and induction of uterine contractions (de Groot et al., 1998; Hill, 2005). As a result, the pharmacological properties of ergot alkaloids in monogastrics are relatively well understood. This knowledge can be applied to the post-ruminal behavior of the compounds in ruminants.

Via examination of the pKa values for various forms of ergot alkaloids in comparison to the pH gradients in the lower intestinal tract, Hill (2005) determined that lysergic acid would not be passively transported across the intestinal epithelium due to being in the ionic form. While more basic ergot alkaloids (ergopeptines and ergolines) are likely to be found in equimolar ratios of ionic and non-ionic forms in the intestinal lumen, and therefore may be passively transported from the intestine into the blood (Hill, 2005). Oral dosing of purified alkaloids in man showed that ergoline alkaloids (i.e. lysergic acid amide and nicergolin) are transported into the blood at 20+ times higher levels than ergopeptine alkaloids (Eckert et al., 1978). Eckert et al. (1978) proposed that the lower transport of ergot peptide alkaloids was in part due to their having both hydrophilic and lipophilic properties. This may relate to the greater potential for toxicity of ergoline alkaloids seen in many studies.

Another factor affecting absorption of ergot alkaloids is their solubility in the intestinal tract. Ergopeptine alkaloids are virtually insoluble, whereas alkaloids with lower molecular weights (clavine and ergoline alkaloids) have a solubility more than 20 times higher (Hill, 2005). Ergotamine received attention from the medical community despite its low solubility due to its ability to relieve migraine headaches (Sargent et al., 1988). In humans therapeutic doses of ergotamine must be at least 0.6mg/kg\textsuperscript{75} to be effective (Sargent et al., 1988). Hill (2005) determined that this would be similar to a 300kg steer with a daily consumption of 2.5\% of its body weight in tall fescue with 800ng/g of ergovaline.
2.5.1.2) Ruminants

Ergot alkaloids induce the symptoms of fescue toxicosis in cattle at much lower levels than in rats and mice. It has been postulated that this is due in part to the smaller metabolic size of cattle (Settivari et al., 2008b). In addition, the longer retention time of feed in the ruminant digestive system may cause more complete extraction of the alkaloids from the feed (Spiers et al., 2005a). Moyer et al. (1993) found evidence for the degradation of 95% of administered ergonovine and 88% of ergovaline, in the liquid phase, after 48h in vitro digestion. This provides evidence for the chemical conversion of dietary ergot alkaloids as a result of degradation by ruminal microbes in the gastrointestinal tract. Though an increase in ergovaline in the insoluble portion was reported, this was attributed to sequestration of the alkaloid by microorganisms (Moyer et al., 1993).

In vitro experiments with ruminal, omasal, and reticular tissue samples from endophyte naive ewes have been used to examine the transport of various ergot alkaloids. Parabiotic chambers were used to evaluate transport by comparing serosal side concentrations of the tissue types after addition of the alkaloid mixture to the mucosal side (Hill et al., 2001). Ergoline alkaloids were reported to be transported at higher rates for all tissue types as compared to ergopeptine alkaloids. Lysergic acid had the greatest serosal side concentration across all tissues, and was highest in ruminal tissue. Ergonovine was the least transported of the tested ergot alkaloids (Hill et al., 2001). Stuedemann et al., (1998) believed that the majority of absorption in the ruminant must be ergoline alkaloids in the foregut due to the short amount of time observed between intake of E+ fescue and excretion of ergoline in the urine. Hill et al. (2001) reported that ergoline alkaloids are primarily absorbed from the ruminant forestomachs, and that the mechanism involves active transport of the ionic form of the alkaloids. This research gives a base for understanding ergot alkaloid absorption in the ruminant. However, the in vivo absorption of ergot alkaloids is affected by ruminal passage rates, liberation of alkaloids from the feed, interaction of
alkaloids with tissue surfaces, and the quantities of alkaloids ingested. As such, it is difficult to model or predict the absorption of alkaloids by individual animals.

2.5.2) Blood Clearance

Direct injection of ergovaline into the jugular of several species has been used to examine toxicokinetics and blood clearance. In horses, rapid disappearance of ergovaline was observed within 10 minutes after a 15µg/kg injection resulting in a half-life of 56.83 ± 13.48min (Bony et al., 2001). The limit of quantification (LOQ) was reached after 150min. These findings are in agreement with studies in sheep (Jaussaud et al., 1998) and goats (Durix et al., 1999) which have shown plasma clearance rates of 0.020 L/min kg⁻¹ and 0.34L/min kg⁻¹ respectively. These experiments also produced the rapid decline in plasma ergovaline levels, with the LOQ reached after 1h. As these studies only measured ergovaline, it is unknown whether the ergovaline is removed, transformed into other alkaloid(s), or broken down to metabolites.

2.5.3) Hepatic Metabolism

The liver is the primary detoxification organ in the body. Consumption of ergot alkaloids can significantly alter enzyme and gene expression in hepatic tissues (Settivari et al., 2006; Brown et al., 2009). This includes an increase in transcription of genes related to detoxification. Minimal research has been conducted on the hepatic metabolism and detoxification of ergot alkaloids in ruminants. However, that which has been reported mirrors the data from rat and mouse models. Studies examining ergot alkaloid metabolism in rats (Peyronneau et al., 1994) and human liver (Ball et al., 1992) suggest that cytochrome P450s, specifically CYP3A, is primarily responsible for the metabolism of ergot alkaloids during detoxification. Similar results have been reported for beef liver microsomes incubated with ergovaline and CYP3A (Moubarak and Rosenkrans, 2000). Though no upregulation of CYP3A has been reported in mouse (Duringer et al., 2005), rat (Moubarak et al., 2003) or bovine (Moubarak and Rosenkrans, 2000) liver tissue during incubation with ergot
alkaloids. This may be an effect of experimental design, as mice (Settivari et al., 2008b) and sheep (Zanzalari et al., 1989) fed E+ fescue seed were reported to have elevated hepatic CYP enzyme expression.

2.5.4) Retention and Bioaccumulation

In vitro experiments examining the contractile response of veins and arteries to ergot alkaloids have provided some evidence of bioaccumulation of ergovaline in the vasculature of animals. Dyer (1993) reported that bovine uterine and umbilical arteries maintained a contractile response to ergovaline for more than three hours. Similarly, bovine lateral saphenous veins (LSV) exhibited a contractile response lasting over 90 minutes after a single addition of ergovaline (Klotz et al., 2007). Tissue strips from the reticulum also exhibited prolonged contraction after stimulation with ergotamine (1.7 x 10^{-6} M), with no evidence of reduction after one hour (Poole et al., 2009).

Klotz et al. (2009) further analyzed bovine LSV treated with differing numbers of exposures to ergovaline and lysergic acid for accumulated levels of the ergot alkaloids. They reported that increasing exposures did not alter the level of lysergic acid recovered from the veins, however, increased numbers of exposures to ergovaline did cause a significant increase in ergovaline concentration measured in veins (Klotz et al., 2009). Further, reduced contractile sensitivity and lumen area of LSV was seen up to 42d after removal of steers from endophyte infected pastures (Bussard, 2012) indicating that accumulation occurs in tissues following grazing of endophyte-infected fescue. Moreover, Realini et al. (2005) reported detection of ergot alkaloids in the adipose of animals finished on endophyte-infected tall fescue, indicating that retention of alkaloids occurs in grazing animals, and may affect meat quality.

Recently, Mulac et al. (2012) showed that ergot alkaloids are able to cross the blood-brain barrier, possibly by active transport. It was also reported that ergocristinine accumulated in cell and altered barrier function (Mulac et al., 2012). If barrier function is affected in other tissues, such as the GIT epithelium,
this may provide the route of entry for alkaloids into the circulatory and lymphatic systems.

2.5.5) Excretion

Eckert et al. (1978) reviewed a number of papers and concluded that the excretion route of ergot alkaloids in non-ruminants depended on the molecular weight of the alkaloid. Ergot alkaloids <350Da (primarily ergoline alkaloids) were excreted in the urine, >450Da (primarily ergopeptine alkaloids) were excreted in the bile, and those with a molecular weight between 350Da and 450Da were excreted in both bile and urine. Stuedemann et al. (1998) studied excretion of ergot alkaloids in Angus steers grazing E+ pastures and found that 95% of excreted alkaloids were found in the urine, with 5% in the bile. This would suggest that the majority of circulating alkaloids in cattle are ergoline alkaloids.

Hill et al. (2001) proposed three possibilities to account for the high levels of excreted ergoline in comparison to the high levels of ergopeptine in tall fescue plants: alkaloids are 1) absorbed as ergopeptides and converted from the ergopeptine form prior to excretion, 2) absorbed primarily in the ergoline form, or 3) absorbed in both forms and metabolized to ergoline prior to excretion. Crossbred wethers fed tall fescue straw excreted 35% of dietary ergovaline in the feces (no detectable amount in the urine) and 248% of dietary lysergic acid was recovered in the feces and urine (De Lorme et al., 2007). This, combined with the research described above by Moyer et al. (1993) indicates that dietary ergot alkaloids are likely converted to lysergic acid prior to absorption and excretion.

2.6) Observable Symptoms

The problems associated with fescue toxicosis can be classified into two major categories, observable symptoms and physiological changes. The primary indicators of fescue toxicosis include reduced weight gain, decreased milk production, and a reduction in reproductive efficiency (Wagner, 2008). These have been separated into four areas: 1) Summer Slump, 2) Fat Necrosis, 3) Fescue Foot, and 4) Reproductive Effects. In the warmer parts of the year,
summer slump is more prevalent. While in fall and winter months, the symptoms of fescue foot are the most common.

2.6.1) Summer Slump

Summer slump has been called the “most common and economically significant” effect of fescue toxicosis in cattle (Spiers et al., 2005b). The visible signs of summer slump are diverse, and include poor growth, rough hair coat due to retained winter hair, elevated body temperature, increased respiration rate, increased time spent in shade or water, and excessive salivation (Wagner, 2008). Many of these symptoms are exacerbated by high environmental temperatures (Spiers et al., 2005b).

In the 1970s researchers noted that animals grazing fescue pastures had reduced gains; developing the rule of thumb that a 10% increase in endophyte infection level corresponded to a 0.045kg/d decrease in ADG (Stuedemann and Hoveland, 1988). In the 1980s studies showed that low endophyte pastures could increase the ADG of animals previously grazing endophyte infected areas (Stuedemann and Hoveland, 1988), indicating that summer slump is endophyte related.

Animals grazing infected fescue are 8-17% more likely to seek shade or stand in water (Bond et al., 1984). Evidence of increased standing in water or wallowing is often seen in mud accumulation on the coat of cattle grazing pastures with high endophyte levels. In addition, cattle grazing endophyte infected pastures are more likely to graze at night (Stuedemann et al., 1985b). Researchers believe that this alteration in behavior is primarily due to the increased body temperature of these animals, as standing in shade and water represent efforts by cattle to reduce heat stress.

2.6.1.1) Alteration of Intake

The behavioral changes reported in cattle grazing infected tall fescue can result in as much as 20% less time spent grazing, and a reduction in intake of 10-50% (Bond et al., 1984; Stuedemann and Hoveland, 1988). This reduction in
intake is believed to be the primary cause of the reduced weight gain seen in these animals. Inhibition of 5-HT receptor uptake increases satiety (Simansky, 1995a). Ergot alkaloids act as agonists on serotonergic receptors (Dyer, 1993) which can depress intake, most likely through increasing satiety (Simansky, 1995b). Administration of a dopamine (D2) and serotonergic (5-HT) receptor antagonist (metoclopramide) increased time spent grazing by Angus steers on endophyte infected tall fescue pastures (Lipham et al., 1989). Metoclopramide acts on serotonergic (5-HT) receptors to increase gut motility and gastric emptying (Talley, 1992). This may reduce satiety in the treated steers, counteracting the intake depression resulting from alkaloid ingestion, but is not likely to be a cost-effective solution in grazing situations.

In addition, there is evidence that tall fescue is less palatable than other forages, which may combine with effects on satiety to result in the observed reduction in intake. Fribourg et al., (1991) found that steers preferred clover over fescue in pastures with endophyte infected fescue, but preferentially grazed fescue in uninfected pastures. Likewise, heifers offered a choice of diets containing E+ or E- seed chose to consume the E- diet (Garner and Cornell, 1987). Rabbits have also been shown to choose uninfected or novel endophyte fescues over wild-type endophyte infected tall fescue (Panaccione et al., 2006). Sheep have been reported to alter rate of intake of forage in relation to physical structure with broad-leaved species > temperate grasses > tropical grasses (Wilman et al., 1996). This may be related to ease of leaf breakage and vein orientation. Wilman et al. (1996) found that tall fescue had intermediate rate of intake as compared to other species; though they did not indicate if their tall fescue was endophyte infected, which has been historically shown to affect intake as discussed above. A reduction in intake can affect digestibility of feed, organ weight, metabolism, and energy balance. These are discussed further below.
2.6.1.2) Effects on Growth

Compilation of growth data from tall fescue studies over a 13 year period showed that increasing endophyte levels was linearly related to decreasing average daily gain in beef steers (Thompson et al., 1993). While early research correlated a 45g/d decrease in ADG with a 10% increase in infection level (Crawford et al., 1989), the compilation of research showed that endophyte level accounted for only a portion of the variation in growth rate (Thompson et al., 1993). Average daily gain was increased in Angus steers grazing endophyte infected tall fescue with metoclopramide (Lipham et al., 1989). Metoclopramide is a D2 and 5-HT receptor antagonist, which is discussed in more detail below.

Weaning weight of calves from endophyte infected tall fescue pastures has been reported to be significantly lower for cow-calf pairs on endophyte infected pastures (Schmidt and Osborn, 1993b; Paterson et al., 1995; Watson et al., 2004). This is related to a reduced average daily gain for these calves (Essig et al., 1989; Forcherio et al., 1992; Schmidt and Osborn, 1993a). These observed low weaning weights are likely due to reduced milk production by the cows (as discussed below), but may also be caused by decreased intake by the calves themselves, as creep feeding can significantly improve calf gains (Tarr et al., 1994).

The reduced gain seen while cattle are on pasture does not carry over to the feedlot. Crossbred beef steers previously grazed in E+ pastures had higher daily gains in a feedlot study than steers previously grazing E- pastures (Piper et al., 1987). Similarly, Angus steers backgrounded on pastures with endophyte infected fescue showed equal or greater feed:gain as compared to steers from uninfected fescue pastures during a feedlot study (Cole et al., 1987). These studies indicate that after removal from endophyte infected fescue animals may have compensatory gain and thus ingestion of ergot alkaloids has no long-term effect on growth. Steers subjected to intake restriction during the growing phase, then re-fed, have been shown to have decreased maintenance requirements (Sainz and Oltjen, 1994). Cole (1987) and Lusby (1990) found that steers from E- and E+ pastures had the largest differences in gain during the first 7-weeks on
feed and concluded that the improved feed efficiency of animals from endophyte infected pastures was not a permanent effect. This 7-week time frame correlates to recent research indicating the vasoconstriction is maintained for at least 42-d following removal from endophyte infected pastures (Bussard, 2012).

Some research has focused on the possibility that the observed reduced growth rates are related to alteration of hormones. Christopher et al. (1990) found a decreased level of circulating growth hormone (GH), while Bolt et al. (1987) and Lipham et al. (1989) saw no difference in GH levels. Numerous factors can alter GH secretion including physiological stress, as well as α-adrenergic agonism, and dopamine receptor agonists (Greenspan and Gardner, 2004). All of which occur when animals consume endophyte infected tall fescue, as discussed in more detail below.

2.6.2) Fescue Foot

The swelling and lameness associated with fescue foot are correlated to alkaloid induced constriction of the blood vessels (Garner and Cornell, 1978), primarily in the hind feet (Thompson and Stuedemann, 1993). Swelling, soreness, and skin discoloration have been observed as quickly as 3 to 7 days after cattle begin grazing endophyte infected fescue (Bush et al., 1979). Edema and vessel constriction, as discussed below, cause a reduction in blood flow to the extremities that results in necrosis of the tissue due to dry gangrene (Bush et al., 1979).

The vasoconstriction caused by the ergot alkaloids occurs in conjunction with damage to vessel lining cells and enhanced blood clotting (Oliver, 2005). Examination of the limbs of affected animals showed that 50% of animals had swollen arteriole walls, reducing lumen size (Garner and Cornell, 1978). Edema and hemorrhaging of tissue were also reported, as were thrombi that plugged the blood vessels (Garner and Cornell, 1978). External symptoms of fescue foot include swelling and reddening of the coronary band, arching of the back, and continual shifting of weight from one limb to another (Spiers et al., 2005b).
Fescue foot generally occurs one to three weeks after introducing animals to E+ fescue during colder temperatures.

In cold climates, vasoconstriction naturally occurs in order to aid in thermoregulation (Sharf, 2008). Woods et al (1966) concluded that the combination of this natural vasoconstriction with ergot alkaloids induced vasoconstriction would reduce blood flow sufficiently to induce gangrenous conditions in the extremities. If allowed to progress, the necrotic tissue will be sloughed, resulting in the loss of ears, tail switch, and/or hooves seen in severe cases of fescue toxicosis.

2.6.3) Fat Necrosis

Fat necrosis, also known as lipomatosis, is characterized by hard masses in the mesenteric fat surrounding the intestine, between the abomasum and rectum (Bush et al., 1979). Occurrence of fat necrosis is believed to be correlated to long term exposure to endophyte infected tall fescue and/or consumption of very high quantities of alkaloids, as lipomatosis occurs most frequently in cattle grazing endophyte-infected fescue pastures with high levels of nitrogen fertilization (Stuedemann et al., 1985a) and infection levels greater than 65% (Smith et al., 2004). This may be due to increased nutrient (i.e. nitrogen) availability leading to elevated concentrations of alkaloids in plant tissues (Rottinghaus et al., 1991). Lipomatosis can cause clinical disease, as well as digestive and reproductive problems. The fat masses take up large volumes of space in the abdominal cavity, resulting in compression and/or obstruction of internal organs (Bush et al., 1979; Kahn and Line, 2005).

Necrotic fat deposits can be identified as hard, caseous lesions within normal fat depots that are often encapsulated by fibrous connective tissue (Bush et al., 1979; Rumsey et al., 1979). They may also exhibit necrotizing steatitis and saponification (Smith et al., 2004). Necrotic fat also tends to be a darker yellow color than normal fat and may have chalky white or orange sections (Bush et al., 1979). Rumsey et al. (1979) found that the chemical composition of necrotic fat deposits was different from normal fat in cattle grazing E+ fescue. Necrotic
samples were higher in protein, ash, and cholesterol, and lower in ether extract than normal fat samples. Townsend et al. (1987) found fat deposits in cattle grazing E+ fescue had a higher percentage of saturated fatty acids. Realini et al (2005) also reported altered adipose composition, including increased saturated fatty acids and reduced monounsaturated fatty acids and conjugated linoleic acids in cattle finished on tall fescue. While these cattle had no change in fat depth or marbling (Realini et al., 2005), swine treated with bromocryptine had reduced back fat thickness, as well as reduced lipid synthesis (Cincotta et al., 1989).

Smith et al. (2004) found histological changes in the adipocytes, including thickening of cell borders, basophilic granular filling, and dissolution of nuclei. It is possible that these compositional and morphological changes cause the adipose to be recognized as foreign tissue in the body. This could explain the appearance of fibroplasia and collagen found in necrotic fat lesions by Hoflund et al (1953), as well as the increased nitrogen observed by Rumsey et al. (1979). It is likely that ergot alkaloids are altering fat metabolism in adipocytes even when necrotic lesions are not observed.

While the exact cause of lipomatosis in relation to fescue consumption is unknown, it has been linked to cattle grazing pastures with high levels of nitrogen fertilization (Stuedemann et al., 1985a; Thompson and Stuedemann, 1993; Bacon, 1995). Numerous factors can result in alteration of adipose composition, including pancreatitis, hyperthermia, and a dietary increase in long-chain saturated fatty acids (Rumsey et al., 1979). Total LCFA quantities are increased in intramuscular fat of animals raised solely on grasses (Rumsey et al., 1972; Westerling and Hedrick, 1979). However, the fatty acid composition of fescue is not significantly different from that of other grasses (Clapham et al., 2005). Nor is endophyte infected tall fescue (Kentucky 31) significantly different in fatty acid composition as compared to other strains of tall fescue (Dierking et al., 2010). Removal of cattle from endophyte-infected pastures or dilution of intake by supplying legume or other grass pasture has been shown to promote a slow reduction in the size of necrotic fat masses (Kahn and Line, 2005).
2.6.4) Reproductive Effects

2.6.4.1) Effects on Cows

A major concern to producers is the reduction in conception rates in cows grazing infected tall fescue. Conception rates can be reduced by as much as 46% in animals grazing endophyte infected tall fescue (Burke et al., 2001). Along with reduced conception rates, calving rates are also seen to decline by 19 to 41% in cattle grazing infected fescue (Porter and Thompson, 1992). Some of the mechanisms that may be responsible for the decrease in these rates have been investigated. Progesterone levels have been shown to be reduced in weanling heifers grazing infected fescue (Mahmood et al., 1994). This reduction in progesterone is believed to be a sign of abnormal luteal function.

The size and structure of the corpus luteum (CL) in cattle has been shown to be altered by consumption of infected fescue. Heifers consuming a diet including infected tall fescue seed showed a reduced CL diameter, reduced size of the dominant follicle, and fewer large follicles than those supplemented with uninfected seed (Burke et al., 2001). These follicular changes seem to occur with no alteration in plasma levels of FSH (Browning et al., 1998b). Additionally, the CLs of heifers grazing endophyte infected fescue had more mitochondria, lipid droplets, and secretory granules than those grazing uninfected fescue (Ahmed et al., 1990). These studies suggest that ergot alkaloids cause an alteration in the development and cellularity of CLs.

Microarray analysis of luteal tissue from cattle consuming endophyte infected hay showed 598 genes and expressed sequenced tags (EST) that were down-regulated and 56 genes and ESTs up-regulated relative to cows on an endophyte free hay (Jones et al., 2004a). This alteration of gene expression is likely the causative agent for the physiological alteration in luteal function seen in cattle consuming ergot alkaloids. Heifers in high endophyte fescue pastures exhibited acyclic hormone levels, shortened luteal phases, and less estrous activity as compared to heifers grazing low endophyte fescue (Mahmood et al., 1994). Further evidence of luteal dysfunction can be seen in the lowered plasma
luteinizing hormone (LH) concentration and increased prostaglandin F2α metabolite (PGFM) secretion in cows challenged with ergonovine and ergotamine injections (Browning et al., 1998b).

An additional theory focuses on the oxytocic action of ergot alkaloids to stimulate strong, sustained uterine contractions (Aleck and Burn, 1927). Ergot alkaloids were used in human obstetrics to induce labor until the early 1800s. Saameli (1978) proposed that the increased embryonic mortality seen with fescue toxicosis (McEvoy et al., 2001) could be attributed to this increase in uterine contractions.

Consumption of infected fescue does not appear to increase the incidence of dystocia or lengthen the calving interval (Brown et al., 1992). Some research has shown no effect on birth weight of calves to cows grazing endophyte infected fescue (Schmidt et al., 1986; Waller et al., 2001), while others have seen a reduction in birth weights related to grazing high endophyte pastures (Smith et al., 1975; Bolt et al., 1989; Watson et al., 2004). It is possible that these differences are related to level of endophyte infection, plane of nutrition, or breed of cattle, and not wholly due to the toxic effects of ergot alkaloids in endophyte infected fescue.

Agalactia is the most common problem in cows resulting from fescue toxicosis. Serum prolactin levels are depressed by ergot alkaloids (as discussed below), resulting in reduced milk production. This is of particular importance to cow-calf operations and dairies, as milk production and quality have been shown to account for 40% of the variance in d-205 weights of calves (Robison et al., 1978). Milk production has been shown to decline 20-50% in Angus, Brahman, and Simmental cows grazing infected fescue compared with cows grazing orchard grass (Peters et al., 1992; Brown et al., 1993; Brown et al., 1996). Similarly, milk fat also declines when cows graze infected fescue, while milk protein is unchanged (1993; Brown et al., 1996). This may be related to fat metabolism as mentioned above. The effect of alkaloid ingestion on milk production is only present short-term, as cows grazing fescue during the pre-
partum period have reduced prolactin without a decrease in milk production postpartum if switched to an alternate forage (Bernard et al., 1993).

2.6.4.1) Effects on Bulls

The majority of reproductive research has focused on the effects of ergot alkaloids on the cow, as most research in bulls has shown little to no alteration in sperm due to infected fescue consumption. Evans et al (1988) found no changes in development or size of the testes and epididymis, or sperm production capacity, in Holstein bull calves. Research with beef bulls showed no alteration in sperm motility or morphology when animals grazed infected fescue pastures (Jones et al., 2004c; Schuenemann et al., 2005) nor any change in sexual behavior (Mays, 2005). Looper et al. (2009) did see evidence of decreased motility in sperm from Brahman influenced bulls grazing infected fescue in heat stress situations. During the months of July and August sperm from these bulls had decreased width of head oscillations and a reduction in straight line velocity. More recent research has shown that ergot alkaloids have a direct effect on spermatozoa to reduce motility (Page, 2011). Sperm from yearling beef bulls grazing infected fescue pastures had reduced cleavage rates, but those embryos that cleaved had no difference in percentage that developed into the eight-cell or blastocyst stage (Schuenemann et al., 2005). While these studies may indicate an effect on bull reproductive performance due to ergot alkaloids, these are likely less significant to reproductive loss than the effects on cows. Ultimately, ergot alkaloids affect numerous aspects and stages of the reproductive cycle, and it is likely that a combination of these effects is responsible for the reduction in reproductive efficiency seen in animals grazing infected fescue.

2.7) Physiological Causes

2.7.1) Biogenic Amine Receptors

Due to the structural similarity between ergot alkaloids and biogenic amines such as epinephrine, dopamine, and serotonin, a variety of receptors are known to be affected by ergot alkaloids (Oliver et al., 1993; Thompson and
Stuedemann, 1993; 1998). It is the downstream effects following ergot alkaloid activation of these receptors that results in the classic symptoms and problems associated with fescue toxicosis (Figure 2.2). Ultimately, it is the combination of a variety of alkaloids acting on several types of receptors in numerous tissues that cause the wide range of physiological changes seen during fescue toxicosis.

Solomons et al. (1989) found that both \( \alpha_1 \)- and \( \alpha_2 \)-adrenergic receptors are present on bovine dorsal pedal vein. Stimulation of these alpha-adrenergic receptors leads to the characteristic vasoconstrictive effect of ergot alkaloids (Leibowitz, 1975; Oliver, 1997; Oliver and Fletcher, 2005). Research has shown that the agonist effect of many ergot alkaloids is greater on \( \alpha-2 \) as compared to \( \alpha-1 \) receptors (McPherson and Beart, 1983; McPherson, 1984; Oliver, 1997). Oliver et al (1994) showed that saphenous veins from animals grazing E+ and E-pastures had no difference in contractile response to phenlyephrine, indicating that activity of \( \alpha-1 \) receptors are not affected by ergot alkaloid intake. However, veins from animals grazing E+ pastures did exhibit a higher contractile response to an \( \alpha-2 \) agonist (BHT-920) (Oliver et al., 1994). Loline alkaloids have been shown to have minimal vasoconstrictive activity in laboratory settings (Solomons et al., 1989; Oliver et al., 1993) and are believed to act through mechanisms other than alpha-adrenergic receptors.

Ergopeptine alkaloids have also been shown to act on serotonin-2 receptors. Stimulation of these receptors can affect satiety (Simansky, 1995b), gut motility (Talley, 1992), and thermoregulation (Gudelsky et al., 1986). Serotonin acts on the hypothalamic satiety center, to suppress appetite (Rossi-Fanelli and Cangiano, 1991; Simansky, 1995c). Ergot alkaloids may act similarly, resulting in the reduced intake often observed during fescue toxicosis. Experiments with rat tail arteries and varied concentrations of ergovaline and ergotamine showed that these ergopeptine alkaloids act as partial agonists to 5-HT\(_{2A}\) receptors (Schoning et al., 2001). It was also noted that ergovaline may bind in an irreversible manner, as evidenced by its ability to reach the maximum contractile response of a full agonist, and the inability of addition of the full agonist or wash-out to reduce the contraction (Schoning et al., 2001). During
experiments examining in vitro arterial vasoconstriction, Dyer (1993) found that ketanserin, a 5-HT\textsubscript{2} antagonist, and phenoxybenzamine, an \(\alpha\)-adrenergic and 5-HT receptor blocker, were able to reduce the contractile ability of ergovaline to a greater extent than selective \(\alpha\)-adrenoceptor antagonists, prazosin and phentolamine. These experiments suggest that ergovaline acts more strongly on 5-HT receptors, than \(\alpha\)-adrenergic receptors.

D2 dopamine receptors can also be stimulated by ergot alkaloids and this stimulation likely contributes to their toxic effects (Dyer, 1993; Larson et al., 1994). Agonist action of ergot alkaloids on D2 dopamine receptors results in a reduction in prolactin secretion by the anterior pituitary. Schillo et al. (1988) found a significant reduction in both circulating and releasable levels of prolactin in cattle that had grazed endophyte infected fescue pastures. Cultured pituitary cells treated with ergocryptine had reduced prolactin gene transcription (Maurer, 1981), which may indicate a reduction in PRL production. The decrease in circulating prolactin is the underlying cause of agalactia seen in cows and mares grazing E+ fescue, as prolactin regulates milk synthesis and secretion (Freeman et al., 2000). Domperidone, spiperone, and metoclopramide are D2 receptor antagonists that have been used to alleviate the depressed PRL levels and agalactia in cattle grazing E+ fescue (Bolt et al., 1987; Lipham et al., 1989; Samford-Grigsby et al., 1997). As with \(\alpha\)-adrenergic receptors, loline alkaloids do not bind to D2 receptors (Larson et al., 1999) and do not reduce prolactin secretion (Strickland et al., 1992).

2.7.2) Vasoconstriction

Vasoconstriction is one of the classic effects of endophyte infected tall fescue consumption. Garner and Cornell (1978), found that in dissected limbs of affected cattle, arterioles were swollen, reducing lumen area. These limbs and blood vessels also showed evidence of edema, hemorrhage, and increased incidence of thrombi plugging the smaller vessels (Garner and Cornell, 1978). Vasoconstriction occurs quickly, as narrowing of the lumen of the caudal artery in
Angus-cross heifers can be seen four hours after feeding a diet containing endophyte infected fescue (Aiken et al., 2007).

In vitro studies have been conducted to evaluate the strength of different alkaloids in causing vasoconstriction. Solomons et al. (1989) found that ergotamine was a strong vasoconstrictor, while agroclavine and ergosine had significantly weaker effects on bovine dorsal pedal veins. Ergovaline was found to be a highly potent vasoconstrictor in bovine uterine and umbilical arteries (Dyer, 1993). Ergotamine has been shown to have similar vasoconstrictive potencies in both bovine (Klotz et al., 2007) and equine (Abney et al., 1993) lateral saphenous veins (LSV). Lysergic acid and its analogue lysergic acid amide have also been shown to cause constriction of bovine LSV (Oliver et al., 1993; Klotz et al., 2006). Conversely, N-acetyl loline produced minimal venoconstriction and no arterioconstriction on equine (Abney et al., 1993) or bovine vasculature (Klotz et al., 2008).

Experiments with dorsal metatarsal arteries and LSV showed that the contractile response to ergot alkaloids occurs to a higher degree and at lower concentrations in veins than arteries (Oliver et al., 1992; Oliver et al., 1993). Research in canines has suggested that α-2 adrenergic receptors are dominant on veins while α-1 receptors appear to be dominant on arteries (Oliver, 1997). This, in conjunction with the previously discussed findings of stronger agonist action by ergot alkaloids on α-2 receptors (Oliver, 1997), may explain why the ergot alkaloid induced vasoconstriction observed in veins is greater than that observed in arteries.

In addition to the direct vasoconstrictive action of ergot alkaloids, there may also be an increase in circulating levels of angiotensin-II, a potent vasoconstrictor (Oliver and Fletcher, 2005). Research has shown that cattle grazing E+ pastures have elevated levels of angiotensin converting enzyme (ACE) (Oliver, 1997), which generally results from increased levels of angiotensin, which causes vasoconstriction (Greenspan and Gardner, 2004). Further, Al-Tamimi et al. (2007) indicated that endogenous nitric oxide (NO) levels were reduced in rats consuming E+ seed. They further determined that
the addition of a NO donor (molsidomine) to the water of these rats could negate increases in core body temperature. They hypothesized that this was due to the activity of NO as a vasodilator (Moncada and Higgs, 1993). This may indicate that a portion of vasoconstriction resulting from ergot alkaloid consumption is due to inhibition of NO production in vascular epithelium.

2.7.3) Body Temperature Alteration

A number of studies have shown a relationship between infected fescue and increased core body temperature (Neal and Schmidt, 1985b; Boling et al., 1989; Aldrich et al., 1993a; Spiers et al., 2005a). This increase is often in association with a decrease in skin temperature (Browning and Leite-Browning, 1997; Browning, 2000) that is attributed to the α-adrenergic receptor mediated vasoconstriction in subcutaneous areas (Dyer, 1993; Oliver, 1997). Rhodes et al. (1991) measured blood flow to various tissues and found that in animals consuming an E+ diet blood flow to the skin was reduced. Reduced blood flow due to vasoconstriction in subcutaneous tissues has been postulated to result in a reduction in heat loss, thereby contributing to the increased core body temperature. In support of this, Al-Haidary et al. (2001) found that during continuous heat challenge, animals consuming a high level of ergovaline had increased core body temperature as a result of reduced cutaneous heat transfer, rather than increased heat production. Continuous measurement studies in cattle and rats have shown that animals consuming fescue alkaloids exhibit hyperthermia primarily during the dark phase (Aldrich et al., 1993b; Spiers et al., 1994; Spiers et al., 1995). The consensus among researchers is that a reduction in cutaneous heat loss due to inability to increase peripheral blood flow in heat stress situations causes the increased core body temperature seen in cattle suffering from fescue toxicosis. Such a reduction in peripheral blood flow and cutaneous heat loss is associated with a reduction in skin surface temperature in humans (Charkoudian, 2003). Purified ergovaline, administered intravenously, reduced skin temperature, and induced heat stress in wethers and geldings.
(Bony et al., 2001; McLeay et al., 2002). While ergotamine administered to cattle intramuscularly lowered tail skin temperature (Carr and Jacobson, 1969).

Alternatively, research has shown no effect of ergovaline on body temperature or skin vaporization during the winter months (Aldrich et al., 1993a; Stamm et al., 1994). This may signify that ergot alkaloids do not affect the thermoregulative ability of animals unless they are exposed to high ambient temperatures, where heat dissipation is critical to temperature regulation (Browning, 2000). This is further shown by data from rat studies where an increased environmental temperature with E+ feeding resulted in an increase in the daily minimum core body temperature, while there was no change in the daily maximum core temperature (Spiers et al., 2005a). Conversely, rats injected with ergovaline at thermal neutral and cold temperatures exhibited decreased core temperature and metabolic rate (Spiers et al., 1995; Zhang et al., 2002). However, it is not certain that this reduction in metabolic rate is not a result of suppression of shivering and other thermogenic processes (Spiers et al., 1995).

In order to better understand the underlying mechanism by which E+ fescue causes hyperthermia in cattle, researchers have used injection of individual alkaloids to mimic the effects of long-term grazing of infected pastures. Ergovaline (Al-Haidary et al., 1995) and ergotamine (Osborn et al., 1992) have also been shown to increase core temperature and decrease skin temperature. Whereas ergotamine tartrate increased rectal temperature and decreased skin temperature in Holsteins (Carr and Jacobson, 1969), but only decreased skin temperature in Angus, Brahman, and Herefords (Browning et al., 1998a; Browning, 2000).

As described above, ergot alkaloids act as partial agonists on 5-HT$_2$ receptors. 5-HT receptors have been shown to regulate thermal balance in the hypothalamus (Lin et al., 1983). Experiments with rats showed that 5-HT$_2$ receptor agonists resulted in hyperthermia, while 5-HT$_{1A}$ agonism led to hypothermia (Gudelsky et al., 1986). Administration of 5-HT agonists in rats also resulted in increased metabolic rates (Lin et al., 1983; Bovetto and Richard, 1995). This data indicates that some of the elevation in body temperature seen
in cattle consuming endophyte infected tall fescue may be due to effects on the hypothalamic thermal regulatory center and increases in basal metabolism.

2.7.4) Cardiovascular Effects

Increased blood pressure has been noted in some studies of ergot alkaloid effects. Research conducted by intravenous injection of alkaloids tends to show this increase (Browning and Leite-Browning, 1997; Browning, 2000; McLeay et al., 2002). While studies that use ground E+ fescue seed or examine animals grazing high endophyte pastures do not see an increase in blood pressure (Rhodes et al., 1991; Aiken et al., 2007). Therefore, increased blood pressure may be an artifact of infusion and not directly caused by the ergot alkaloids. Numerous studies have shown a reduction in heart rate with consumption of infected fescue (Osborn et al., 1992; Browning and Leite-Browning, 1997; Aiken et al., 2007). This reduction is often seen without the increase in blood pressure noted above. It has been postulated that the heart rate of animals is not directly affected by the alkaloids, but rather is reduced in response to the vasoconstriction, allowing for the maintenance of a constant blood pressure (Oliver, 2005). From these data follows another possible reason for the discrepancy in alteration of blood pressure. Blood pressure may be temporally affected; in that vasoconstriction occurs quickly and would result in increased blood pressure that could be attenuated over time due to slower regulation of mechanisms such as reduced cardiac output and heart rate.

Rhodes et al. (1991) reported decreased blood flow to both core and peripheral tissues as a result of E+ fescue consumption. Radio-labeled microspheres showed reduced blood flow to rib and leg skin, as well as the adrenal glands, cerebellum, duodenum, and colon. This reduction in blood flow should result in a concomitant increase in blood flow to other areas. However, blood flow to other tissues remained unchanged. This led to the conclusion that E+ fescue consumption results in a reduced cardiac output in cattle (Rhodes et al., 1991). This is in agreement with previously research by Johnston and Saxena (1978), who measured decrease in cardiac output of cats injected with
ergotamine. They also reported decreased blood flow to the lungs and heart, with increased blood flow to the uterus. As ergotamine causes uterine contractions, the observed increase in blood flow was postulated to be a result of increased nutrient demand due to these contractions (Johnston and Saxena, 1978).

2.7.5) Respiratory Effects

   Respiratory distress (labored breathing and/or shortness of breath) and increased respiration rate are frequently seen in cattle grazing endophyte infected fescue. The majority of cases of respiratory distress are noted at high ambient temperatures, with few at thermoneutral or cold temperatures (Carr and Jacobson, 1969; Walls and Jacobson, 1970; Osborn et al., 1992; McLeay et al., 2002). In horses, animals consuming endophyte infected tall fescue had elevated respiration rates at 5 and 10 minutes post exercise (Webb et al., 2010). This may indicate that animals consuming E+ fescue utilize more energy and require more time to recover to resting levels or have a reduced oxygen carrying capacity. An increase in energy usage for respiration in animals already energy compromised as a result of reduced intake may be a significant secondary contributor to weight loss in these animals.

   Direct effects of ergot alkaloids on lung tissue and blood platelets results in hypoxemia, which leads to an increase in respiration rate in order to sufficiently oxygenate tissues (Oliver, 1997). Cats injected with 5-20μg/kg ergotamine exhibited an 8-22% decrease in venous blood O$_2$ saturation (Johnston and Saxena, 1978). Lung tissue has α- and β-adrenergic receptors (Porcelli and Bergofsky, 1973) as well as seritonergic 5-HT receptors (Sadavongvivad, 1970). Stimulation of serotonergic receptors have been shown to have a bronchoconstrictive effect (Oliver, 1997). The alpha-2 adrenergic receptors present on blood platelets increase their production of throboxane when stimulated by ergot alkaloids, which can cause platelet aggregation (Oliver, 1997). These combine to result in a reduction in blood O$_2$ saturation and tissue oxygenation in animals suffering from fescue toxicosis.
2.7.6) Gastrointestinal Effects

2.7.6.1) Mobility and Passage Rate

Gastro-intestinal tract (GIT) motility can significantly affect passage rate and digestibility of feed (Church, 1988). Both α-adrenergic and serotonergic 5-HT receptors have been implicated in control of GIT motility (Talley, 1992; van Miert et al., 1994) and are acted on by alkaloids present in endophyte infected tall fescue (Oliver et al., 1993; Schoning et al., 2001). In addition, the fat necrosis sometimes seen in association with fescue toxicosis (Smith et al., 2004) and the lolitrem compounds in toxic ryegrass (McLeay et al., 1999) have been reported to alter reticulo-ruminal contraction rates. Intravenous injection of ergotamine and ergovaline also reduced reticulo-ruminal contractions (McLeay and Smith, 2006). Research with steers (Goetsch et al., 1987; Forcherio et al., 1995) and lambs (Aldrich et al., 1993a) reported no change in liquid passage from the rumen. While Hannah et al. (1990) reported increased ruminal liquid outflow rates, but no change in particulate passage rate for E+ fed sheep. Forcherio (1995) also observed no change in particulate passage due to alkaloid ingestion.

Passage rate can also be altered by water intake (Church, 1988). In cattle, level of water intake is controlled by DM intake and ambient temperature (Winchester and Morris, 1956a). As the effects of fescue toxicosis are exacerbated by heat stress, water consumption is likely of increased importance to animals affected by fescue toxicosis. Aldrich et al., (1993b) found that water consumption was increased by heat stress, but not altered by E+ consumption, though DMI was decreased by both elevated temperature and endophyte treatment. In a previous study, water intake relative to DMI was not different between fescue treatments (Aldrich et al., 1993a). This is contrary to research reporting reductions in water intake with E+ treatment, in association with reduced DMI (Aldrich et al., 1993a; Humphry et al., 2002). Conversely, increased water consumption (corrected for BW) has been reported for cattle grazing endophyte infected fescue pastures, with water intake corresponding to increasing environmental temperature (Parish et al., 2003). This increased water
intake has been attributed to the increased water requirements due to excess salivation and increased respiration often seen during fescue toxicosis (Stuedemann and Hoveland, 1988; Osborn et al., 1992). Most research has not separated the effects of environmental temperature, DMI, and alkaloid ingestion when examining water intake. Thus it is difficult to conclude whether water consumption is altered by tall fescue alkaloid ingestion. Changes in water consumption may also relate to alterations of passage rate and feed digestibility during fescue toxicosis. However, as with other factors, DMI, water consumption, and fescue treatments have not been well separated to differentiate between these effects.

2.7.6.2) Digestibility and Nutrient Absorption

Previous research has provided evidence for increased (Schmidt et al., 1982; Goetsch et al., 1987), decreased (Westendorf et al., 1993; Aldrich et al., 1993b; Matthews et al., 2005), and unchanged (Harmon et al., 1991; Forcherio et al., 1995) digestibility of endophyte infected tall fescue hay in cattle. The increased digestibility observed by Goetsh et al., (1987) is likely a function of DMI and not due to alkaloid presence in the feed. Similarly, the 3% increase in IVDMD reported by Schmidt et al., (1982) is not likely to be physiologically significant. Flores et al., (2007) observed no practical difference in in situ digestibility between novel and wild-type endophyte infected tall fescue, indicating that differences in nutrient flux and metabolism are most likely due to indirect effects of the alkaloids, as opposed to alteration of digestion. Further, it is important to remember that the experiments conducted by Harmon et al., (1991), Forcherio et al., (1995) and Flores et al., (2007) were performed at or below thermoneutral temperatures, and the most adverse effects of fescue toxicosis are seen during times of heat stress. Thus changes to digestion may only occur at elevated environmental temperatures.

Limited studies have examined nutrient absorption and blood nutrient concentrations in animals fed E+ fescue. As discussed previously, consumption of endophyte infected tall fescue leads to altered blood flow. When this ischemia
occurs in the GIT, it likely leads to altered nutrient flux (Oliver and Fletcher, 2005). Recent research has shown decreased blood flow to the rumen epithelium as well as decreased VFA absorption (Foote et al., 2012b). Arterial concentrations of butyrate and ammonia N decreased when steers were fed endophyte infected hay (Harmon et al., 1991). No alteration in arterial concentrations of other nutrients (Harmon et al., 1991) have been shown. Matthews et al (2005) reported reduced N retention in steers fed E+ hay as compared to E- and NE controls. However, this is likely a function of reduced intake, as fecal N and urinary N were not different (Matthews et al., 2005). Both of these studies used E+ and E- hay fed at a limited intake, though Matthews et al. observed lower DMI for E+ fed animals. In addition, these experiments were conducted at temperatures at or below thermoneutral. The most significant reductions in intake and growth rate for cattle grazing E+ fescue are seen during times of heat stress (Bond et al., 1984; Stuedemann and Hoveland, 1988), thus any changes to nutrient flux and blood flow may be more apparent at elevated ambient temperatures.

2.7.7) Hepatic Enzyme Alteration

Steers grazing high endophyte pastures had reduced liver weights (10%) at slaughter when compared to steers grazing low endophyte pastures (Brown et al., 2009). Similar results were found in rats consuming diets with E+ tall fescue seed fed both ad libitum (Settivari et al., 2006) and pair fed (Chestnut et al., 1992), indicating that reduced liver weight may not be solely due to reduced energy intake. Settivari (2006) also found that hepatic glycogen stores were depleted in rats fed an E+ diet.

Both direct enzyme measurement and genetic microarray analysis have shown that hepatic enzymatic activity is altered with toxic fescue consumption. Reduced LDH in steers grazing E+ fescue suggests a decreased ability to convert muscle pyruvate to lactate as well as reduced capacity for conversion of lactate to pyruvate for gluconeogenesis (Brown et al., 2009). In contrast, AST content in the liver has been found to be increased with high levels of endophyte
consumption (Brown et al., 2009) which indicates an increased ability for the interconversion of aspartate and OAA in the TCA cycle. At the same time, these steers had increased levels of PEPCK-C, thus allowing for the conclusion that steers grazing HE pastures have an increased ability for gluconeogenesis through increased conversion of aspartate to OAA and subsequently phosphoenolpyruvate. Similarly, rats fed E+ diets ad libitum also showed elevated hepatic levels of genes involved in gluconeogenesis, including PEPCK and fructose-1,6-bisphosphate with reduced feed intake (Settivari et al., 2006). As the animals used by Brown et al. (2009) were on pasture, intake was not recorded, however, animals grazing endophyte-infected fescue typically have reduced intakes. Thus the increase in PEPCK is contrary to research in dairy cows showing that feed restriction reduces PEPCK mRNA expression (Velez and Donkin, 2005). Though the conclusion that gluconeogenesis may be upregulated in animals consuming endophyte-infected fescue is validated by research where animals injected with ergotamine were reported to have elevated blood glucose and reduced circulating insulin levels (Browning et al., 1997).

2.7.8) Effects on Blood Profile

E+ appears to alter bone marrow synthetic activity (Bond et al., 1984; Oliver, 1997). Total counts of neutrophils, lymphocytes, eosinophils, and monocytes were significantly reduced in calves grazing infected tall-fescue pastures for 154 days (Oliver, 1997). Cattle grazing E+ tall fescue for 105 days were found to have reduced levels of monocytes and increased levels of eosinophils (Brown et al., 2009). While a three year study of steers found a decrease in numbers of eosinophils, but an overall increase in total erythrocyte counts (Oliver et al., 2000). Oliver et al. (2000) also noted a decrease in erythrocyte size, along with hypochromic anemia (decreased hemoglobin). Previous research has also shown that hematocrit can be reduced when animals are consuming infected fescue (Thompson and Stuedemann, 1993; Oliver, 1997). Contrary to this, Brown et al. (2009) found no change in packed cell volume, though they did find a 7.1% increase in RBC when steers grazed E+
forage. Oliver et al. (2000) also reported an increase in RBC. The mechanism for this reduction is unknown, as reduced serum prolactin is generally correlated to reduced RBC production (Socolovsky et al., 1998).

2.7.8.1) Blood Lipid Changes

A reduction in serum cholesterol is generally associated with stress due to alteration of lipid metabolism (Realini et al., 2005). In cattle grazing high endophyte fescue, a correlation between reduced serum cholesterol and occurrence of fat necrosis has been observed (Stuedemann et al., 1985a; Stuedemann and Hoveland, 1988). Cincotta et al. (1989) also reported reduced cholesterol in swine implanted with the synthetic ergot alkaloid bromocriptine. Conversely, Brown et al. (2009) observed a decrease in serum cholesterol without any indication of necrotic tissue in the adipose of the GI tract. Serum free fatty acids levels were also increased for cows grazing endophyte-infected fescue (Peters et al., 1992).

2.7.8.2) Immunological Changes

As discussed above, prolactin is decreased in animals grazing E+ fescue. In addition to its role in milk secretion, PRL is a co-factor in humoral immunocompetence regulation (Dawe et al., 1997; Rice et al., 1997). This leads to the premise that animals consuming E+ fescue are immunocompromised due to a decreased ability to produce antibodies. In a short term study, Angus steers grazing high endophyte (70%) tall fescue had reduced antibody response when immunized with tetanus toxoid (Dawe et al., 1997). Similarly, reduced serum hemagglutination titers have been reported in rats fed endophyte-infected fescue seed as compared to those fed uninfected fescue seed (Dew, 1989; Simeone et al., 1998). Response to a lipopolysaccharide challenge was exacerbated by E+ inclusion in the diet of cattle (Filipov et al., 1999). Evidence of long-term immunosuppression has been shown in research with steers grazing E+ tall fescue pastures over a two year period that reported depressed levels of alpha and gamma immunoglobulins (Schultze et al., 1999). In calves from heifers
grazing tall fescue, late weaning has been reported to result in a stronger immune response to vaccination at weaning (Caldwell et al., 2011). Conversely, calves fed E+ diets, showed no alteration of immune response with weaning date (Dew, 1989). Rice et al., (1997) also found no difference in titer levels when steers grazing E- and E+ pastures were injected with lysozome, SRBC, and concanavalin A antigens. Most of these studies used animals in pasture or fed ad libitum, such that E+ animals had lower feed intakes. As reduced protein and energy intake reduces immunocompetence in animals (Galyean et al., 1999), these results may be confounded by nutritional status, and thus not true indictors of ergot alkaloid affects on immune function.

2.7.8.2.1) Copper Status

The long rough haircoats and indication of immunosuppression of cattle grazing infected tall fescue led researchers to examine the copper status of these animals. Copper (Cu) deficiencies can also result in alteration of hair growth and immune function (NRC, 1994; Bonham et al., 2002). A reduction in serum Cu has been reported for cattle grazing infected tall fescue (Coffey et al., 1992; Saker et al., 1998). While other research has shown lowered liver Cu concentrations despite a lack of difference in serum Cu (Stewart et al., 2010). Stabel et al. (1993) found that cattle with a copper deficiency had a reduced antigen response to immune challenge. Beef steers grazing infected tall fescue exhibited lower phagocytic activity by macrophages, reduced major histocompatibility complex II expression, and ceruloplasmin in conjunction with lowered levels of plasma Cu (Saker et al., 1998). These results indicate that a reduction in serum copper levels in animals grazing endophyte infected tall fescue may be responsible for the increased incidence of morbidity in these animals, by compromising immune function (Purdy et al., 1989). This reduction in serum Cu is believed to be due to a reduction in Cu in tall fescue infected with the endophyte (Dennis et al., 1998; Oliver et al., 2000). Copper concentrations were lower in E+ tall fescue plants grown in greenhouses, as well as pastures in both Texas and Virginia (Dennis et al., 1998). Malinowski et al. (2004) examined
the binding of Cu$^{2+}$ by extracellular root exudates of tall fescue, finding that when grown with limited phosphorus, E+ fescue roots have less free copper. This may contribute to the lowered Cu levels in the plant and ultimately the cattle grazing the pastures. Alternatively, the alteration in copper status in animals grazing endophyte infected tall fescue may be related to reduced forage intake, which, as discussed above, results in reduced nutrient intake (including copper). Steers implanted with copper oxide needles while grazing tall fescue pastures showed no significant improvement in serum prolactin levels or grazing performance (Coffey et al., 1992). This indicates that reduced serum copper and the related immunosuppression is a secondary effect of grazing tall fescue and not a causative agent in fescue toxicosis.

2.7.9) Alteration of Energy Metabolism

As mentioned previously, animals consuming endophyte infected fescue exhibit reductions in intake and growth (Bond et al., 1984; Stuedemann and Hoveland, 1988). It is unlikely that this reduction in weight is due solely to reduced intake. The previously discussed changes in organ mass, gene expression, and nutritional and immune stresses can affect energy metabolism, altering nutrient availability and use. Splanchnic tissues account for up to 25% of total body energy expenditure (McBride and Kelly, 1990) and 40-50% of oxygen use (Huntington, 1990). A reduction in intake can reduce splanchnic energy use as a result of decreased visceral mass (Seal and Reynolds, 1993; McLeod and Baldwin, 2000).

Whole body energy use may also be altered by alkaloid consumption. Iason and Murray (1996) found that sheep dosed with plant phenolic compounds had increased whole body energy use and increased urinary energy output. Several studies report that consumption of ergot alkaloids and reduced ME intake may interact to alter energy metabolism in rats consuming endophyte-infected tall fescue (Zhang et al., 2002; Spiers et al., 2005a). However, minimal research has been conducted to examine and separate the effects of reduced energy intake and alkaloid consumption during fescue toxicosis in cattle.
Research in dairy cattle has shown that heat stress and reduced level of intake have discrete effects on milk production and hormone levels (Rhoads et al., 2009), and similar results have also been reported for growing bull calves (O’Brien et al., 2010). In addition, fed rats injected with ergovaline showed acute reductions in metabolic rate that were greater at thermoneutral temperatures than during either heat or cold stress (Spiers et al., 1995; Zhang et al., 2002). The energy cost of thermoregulatory activities of the animals at high and low environmental temperatures (i.e. shivering or panting) may explain the increased metabolic rate at temperatures outside the thermoneutral range (Stanier et al., 1984) and account for the more dramatic reductions in growth seen in cattle during heat stress. These studies indicate that separating the confounding effects of heat and reduced intake from alkaloid ingestion are crucial to understanding the changes that occur during fescue toxicosis.

2.8) Summary

Consumption of endophyte infected tall fescue by cattle results in reduced growth and a myriad of physiological changes. The effects of endophyte-infected tall fescue on livestock have been studied for several decades. However, there are still areas in the research and understanding of the underlying mechanisms of production losses that have not been fully elucidated. The primary effect of alkaloid consumption is a reduction in intake. Thus, most research is confounded by the difference in intake between control animals and those consuming endophyte-infected fescue. In addition, most research relies on animal consumption of infected grass or hay to induce fescue toxicosis. This not only results in differences in forage source between treatments, but also causes variation in the quantity of alkaloids ingested over the course of the experiment due to reduced intake. Co-seeding with a legume and use of supplemental feeds are common amelioration techniques. However, information concerning the ability of an animal to digest and utilize such feedstuffs while ingesting alkaloids from infected tall fescue is minimal. Further, there is a lack of data related to whole body nitrogen and energy metabolism in cattle during fescue toxicosis.
It is these gaps that the research presented in this dissertation aims to fill. In addition, as mentioned previously, much research confounds feed intake, environmental temperature, and level of fescue (alkaloid) ingestion, despite research indicating that these factors have discrete effects on animal performance. Thus, the research presented here attempts to minimize the confounding of these effects, in order to elucidate which changes are attributable to animal ingestion of the alkaloids present in endophyte-infected tall fescue.
Table 2.1: Summary of Proximate Analysis of Tall Fescue

<table>
<thead>
<tr>
<th></th>
<th>Dry Matter (%)</th>
<th>IVDMD&lt;sup&gt;a&lt;/sup&gt; (%)</th>
<th>Crude Protein (%DM)</th>
<th>Crude Fiber (%DM)</th>
<th>NDF&lt;sup&gt;b&lt;/sup&gt; (%DM)</th>
<th>ADF&lt;sup&gt;c&lt;/sup&gt; (%DM)</th>
<th>Ether Extract (%DM)</th>
<th>Ash (%DM)</th>
<th>Cellulose (%DM)</th>
<th>Hemi-cellulose (%DM)</th>
<th>Lignin (%DM)</th>
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</thead>
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<tr>
<td>KY-31 (summer)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>62.7</td>
<td>14.2</td>
<td>58.4</td>
<td>31.4</td>
<td>3.9</td>
<td>8.8</td>
<td>25.5</td>
<td>3.4&lt;sup&gt;y&lt;/sup&gt;</td>
<td></td>
<td></td>
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<tr>
<td>KY-31 (fall)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>55.0</td>
<td>12.6</td>
<td>59.8</td>
<td>31.6</td>
<td>3.5</td>
<td>8.4</td>
<td>28.2</td>
<td>5.6&lt;sup&gt;y&lt;/sup&gt;</td>
<td></td>
<td></td>
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<tr>
<td>KY-31 (March)&lt;sup&gt;e&lt;/sup&gt;</td>
<td>47.6</td>
<td>9.5</td>
<td>37.7</td>
<td>72.6</td>
<td>39.9</td>
<td>2.8</td>
<td>10.5</td>
<td>29.6</td>
<td>32.8</td>
<td>7.3&lt;sup&gt;y&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>KY-31 (May)&lt;sup&gt;e&lt;/sup&gt;</td>
<td>23.6</td>
<td>67.5</td>
<td>10.4</td>
<td>33.7</td>
<td>65.6</td>
<td>36.0</td>
<td>3.0</td>
<td>7.1</td>
<td>29.5</td>
<td>29.7</td>
<td>6.3&lt;sup&gt;y&lt;/sup&gt;</td>
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<tr>
<td>KY-31 (August)&lt;sup&gt;e&lt;/sup&gt;</td>
<td>25.3</td>
<td>10.3</td>
<td>32.2</td>
<td>63.3</td>
<td>38.3</td>
<td>4.4</td>
<td>10.3</td>
<td>27.7</td>
<td>25</td>
<td>7.8&lt;sup&gt;y&lt;/sup&gt;</td>
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<tr>
<td>KY-31 (November)&lt;sup&gt;e&lt;/sup&gt;</td>
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<td>63.3</td>
<td>10.6</td>
<td>32.0</td>
<td>59.8</td>
<td>35.0</td>
<td>2.9</td>
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<td>27.5</td>
<td>24.8</td>
<td>7.6&lt;sup&gt;y&lt;/sup&gt;</td>
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<td>9.4</td>
<td>68.9</td>
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<td>Tall Fescue (E-)&lt;sup&gt;g&lt;/sup&gt;</td>
<td>76.5</td>
<td>23.0</td>
<td>50.6</td>
<td>27.1</td>
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<td>49.0</td>
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<td>17.3</td>
<td>32.1</td>
<td>60.6</td>
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<td>17.6</td>
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<td>72.1</td>
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<td>7.8</td>
<td>36.2</td>
<td>30.4</td>
<td>5.4&lt;sup&gt;z&lt;/sup&gt;</td>
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<tr>
<td>Tall Fescue (May; E-)&lt;sup&gt;j&lt;/sup&gt;</td>
<td>94.2</td>
<td>6.9</td>
<td>72.0</td>
<td>45.8</td>
<td>8.7</td>
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<td></td>
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</tr>
<tr>
<td>KY-31 (May; E+)&lt;sup&gt;j&lt;/sup&gt;</td>
<td>94.4</td>
<td>6.6</td>
<td>75.1</td>
<td>47.1</td>
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<sup>a</sup>In vitro dry matter digestibility; <sup>b</sup>Neutral Detergent Fiber; <sup>c</sup>Acid Detergent Fiber; <sup>d</sup>Barton et al., 1976; <sup>e</sup>Bagley et al., 1983; <sup>f</sup>Bernard et al., 1993; <sup>g</sup>Moyer et al., 1993; <sup>h</sup>Brown et al., 2009; <sup>i</sup>Goetsh et al., 1987; <sup>j</sup>Forcherio et al., 1995; <sup>y</sup>permanganate lignin; <sup>z</sup>acid detergent lignin
Figure 2.1: General Structures of Ergot Alkaloids

- Clavine Alkaloid Structure
- Ergopeptidine Alkaloid Structure
- Loline Alkaloid Structure
- Lysergic Acid
Figure 2.2: Downstream Effect of Ergot Alkaloids on Different Receptor Types

Ergot Alkaloids

- $\alpha_1$- and $\alpha_2$- adrenergic receptors
  - vasoconstriction
  - enhanced blood aggregation

- D2- dopamine receptors
  - anterior pituitary
    - ↓ prolactin secretion
  - hypothalamic thermoregulatory center
    - ↑ body temperature

- serotonin-2 receptors
  - appetite depression
  - bronchio-constriction
CHAPTER 3: EVALUATION OF A RUMINALLY DOSED TALL FESCUE SEED EXTRACT AS A MODEL FOR FESCUE TOXICOSIS IN STEERS

3.1) Introduction

Tall fescue (Lolium arundinaceum ‘Kentucky 31’) is grown on more than 15 million hectares (Thompson et al., 1993) of land in the United States and more than half of these fields are infected with the endophyte Neotyphodium coenophialum (Jones et al., 2004a). While this endophyte provides positive aspects such as drought and heat tolerance to the plant (Settivari et al., 2006), the ergot alkaloids it produces cause health and production problems in animals grazing the infected fescue resulting in negative economic effects for producers (Hoveland, 2007).

The clinical symptoms of fescue toxicosis include reduced feed intake and weight gain, decreased milk production, reductions in reproductive efficiency, tissue necrosis, and a rough hair coat (Wagner, 2008). Physiological signs including increased respiration rate (Browning and Leite-Browning, 1997; Al-Haidary et al., 2001), increased core temperature (Hannah et al., 1990; Rhodes et al., 1991; Aldrich et al., 1993b), and a reduction in serum prolactin can be used to diagnose less severe cases of fescue toxicosis (Oliver, 1997).

As endophyte toxins are concentrated in tall fescue seed, research examining the effects of endophyte infected tall fescue on animal performance often utilizes ground seed added to a basal diet to induce symptoms of toxicosis. Because one of the factors associated with fescue toxicosis is a reduction in feed intake (Rhodes et al., 1991; Aldrich et al., 1993a), achieving a consistent and adequate intake of toxins can be a complication. A more precise method would be to dose the animals with an extract containing the alkaloids found in toxic fescue. However, little research has been done to determine the bioactivity of...

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1 ©Journal of animal Science, published with permission (Appendix B):
such extracts. The goals of these experiments were: 1) To develop a set of standard physiologic measurements to characterize the onset of fescue toxicosis and 2) To determine the ability of a tall fescue seed extract to cause the symptoms of fescue toxicosis in steers.

3.2) Materials and Methods

All procedures involving animals were approved by the University of Kentucky Animal Care and Use Committee. Four Holstein steers (BW = 337 ± 24 kg), surgically fitted with ruminal cannulas (Bar Diamond Inc., Parma, ID), were used in two experiments, both utilizing a two period cross-over experimental design. Each experiment consisted of two, 7-d periods. Each period consisted of two phases that differed in environmental temperature; Phase 1: D1-3 at 22°C and Phase 2: D 4-7 at 32°C. Steers were housed in temperature and humidity controlled rooms and fed once daily at 0800. The basal diet consisted of endophyte-free fescue hay, top dressed with 40 g trace mineralized salt (Table 1). Water was available ad libitum throughout the trial. During the first experiment treatments were ground KY-31 seed from endophyte infected (E+) and uninfected (E-) lots (as determined by ergovaline concentration). During the second experiment seed from these lots was extracted as described below to provide endophyte infected and uninfected extract treatments. Animals were adapted to the basal diet and housing for a minimum of 2 wks prior to beginning the first experiment.

3.2.1) Experiment 1: Ground Fescue Seed

Two randomly selected steers were ruminally dosed with 0.5 kg ground endophyte infected KY-31 fescue seed (E+; 5.3 ppm ergovaline; 3.3 ppm ergovalinine), and two control steers were ruminally dosed with 0.5 kg ground endophyte free KY-31 fescue seed (E-, 0.0 ppm ergovaline and ergovalinine) twice daily (0800 and 1600) during each period. Fescue seed was ground to pass through a 2 mm screen (Model 3 Wiley Mill, Arthur H. Thomas Co., Philadelphia, PA). Environmental temperature was maintained at 22°C during d1
to 3 (phase 1). Beginning on d4, ambient temperature was increased to 32°C (phase 2). During each phase, temperature was held constant, with no 24 h cyclic changes.

Feed intake was measured continuously via feed bunks attached to load cells (LC101-500, Omegadyne, Sunbury, OH) which recorded bunker weight at 1-min intervals. Orts were collected, weighed, and recorded from the previous day prior to each day’s 0800 feeding. After each period, all steers were returned to basal diets and room temperature was returned to 22°C. A minimum of a 3 wk washout was used to eliminate the potential of carry-over effects from the first period. The cross-over period was identical to the first with each steer on the alternate treatment.

At 0900 and 1500 on d 1 through 7 skin surface temperature was measured using an infrared thermometer (Model 42511, Extech Instruments, Waltham, MA). Measurements were taken in the center of a 7.5 x 10cm area over the ribs with the hair removed. Three measurements were taken in each location to provide an average measurement. At each time point, respiration rate was measured by flank movement counts.

During ruminal cannulation, each steer had a radio telemetry device (CorTemp, HQ Inc, Palmetto, FL) placed in the abdomen to continually measure and record core temperature at 1 min intervals. Heart rate was continually measured at 1 min intervals using a telemetry device attached to a heart-girth band (WearLink, Polar Brand, Brooklyn, NY). Room temperature and humidity were recorded throughout the experiment via continuous data-logger (EL-USB-2-LCD, Lascar Electronics, Salisbury, UK). Blood samples were taken via jugular venipuncture on d 1 and 7 of each period, and analyzed for serum prolactin by RIA following the procedures of Bernard et al. (1993) with an intrassay CV of 5.12%.

3.2.2) Experiment 2: Fescue Seed Extract

The animal model, experimental design, and series of measurements validated using ground tall fescue seed was used in two additional periods to
evaluate the efficacy of a fescue seed extract for inducing fescue toxicosis. During this experiment, a digital oscillometric sphygmomanometer was used to determine systolic and diastolic blood pressures using the methods of Browning and Leite-Browning (1997). Diet and feeding of steers in this experiment were as described in Exp. 1, substituting tall fescue seed extracts for ground seed. Extract was produced from the same lots of seed as the ground seed used in Exp. 1 providing extract from endophyte infected tall fescue (EE+) and uninfected tall fescue (EE-). As previously described, blood samples were analyzed for serum prolactin with an intrassay CV for prolactin of 6.78%.

Fescue seed extract was prepared from ground seed packed in an extractor column, utilizing the technique of Fannin et al (2010). Briefly, seed was ground as described above and packed into a 30 cm x 80 cm column, minimizing air spaces and ensuring even compression. The bottom of the column contained approximately 4 cm of glass nuggets covered with an expanded metal screen and a Miracloth filter (Calbiochem, Gibbstown, NJ) to keep the ground seed above the glass nuggets. Approximately 25 kg of seed could be extracted in the column each time. Extraction solution was 80% ethanol and sufficient volume was added to fill the void volume (approximately 38 L) over 6 h. When the solvent front migrated to the bottom of the column, flow was stopped and the seed steeped for a minimum of 12 h. The column was then eluted with 80% ethanol at 2 L/h over a 25 h period into a porcelain container. After elution the ethanol was evaporated by blowing air across the surface. After evaporation, the remaining residue was freeze-dried in the dark, then ground under liquid nitrogen with mortar and pestle. Dried extract was solublized in 80% methanol.

Quantitative determination of ergot alkaloids was performed using ultra performance liquid chromatography/tandem mass spectrometry using an Acquity UPLC®-TQD (Waters, Inc., Milford, MA) as described previously (Foote et al., 2012a). Briefly, 5 µL of the diluted sample was injected (full loop mode) onto an Acquity UPLC® BEH column (C18, 1.7 mm particle size, 2.1 x 100 mm; Waters, Inc.). Separation was accomplished with a linear binary gradient using water with 0.04% NH₄OH (A) and acetonitrile with 0.04% NH₄OH (B) and a constant
flow of 0.50 mL per minute. Gradient program conditions were as follows: initial to 0.6 min – 100% eluent A; at 6.0 min – 10% eluent A/90% eluent B; at 6.1 to 8.5 min – 100% eluent B; at 8.6 to 10 min – 100% eluent A. Detection was accomplished by running the triple-quad mass detector (TQD; Waters, Inc.) in the MS-MS mode following positive electrospray ionization. Concentrations of each alkaloid were determined using a calibration curve with an internal standard (methysergide, 5.0 fmol on column). The calibration curves were linear ($R^2 > 0.97$) within a range of 5 to 250 fmol. Area under the curve values for both the “ine” and “inine” epimers were summed for quantitation as interconversion of the epimers readily occurs in solution.

Ergot alkaloid concentrations for the final product were 102 ppm ergovaline and 60 ppm ergovalinine. This constituted an 81% recovery of alkaloids with a 19-fold increase in concentration. Single dose amounts of ground extract were packaged in cellulose paper and stored in the dark at -5°C to insure stability until ruminal dosing.

3.2.3) Statistical Analysis

The data were analyzed with the Mixed procedure of SAS (SAS Institute, Cary, NC) with individual steer as the experimental unit. Data from each experiment were analyzed separately. Animal and period were considered random effects, while endophyte presence and temperature phase were fixed effects. Data were analyzed for affects of treatment, temperature and the interaction of treatment×temperature. All results are presented as least squares means of all data collected within each temperature phase. Core temperature, heart rate, rate of intake, and meals data were averaged by hour within day prior to analysis. Rate of intake was calculated as the slope of the natural log of feed weight vs. time. Meals were defined as a 10% or greater consumption of current bunk content over a 5min period. All other variables were averaged within day prior to analysis. Sums of squares were partitioned into the main effects of endophyte and temperature as well as their interaction. Prolactin concentrations were analyzed using the Mixed procedure as described above, with animal and
period as random effects, and endophyte and day as fixed effects. In addition, a single tail t-test was performed to determine if prolactin means differed from zero (HO=0). Treatment effects were considered significant at $P \leq 0.05$.

3.3) Results

3.3.1) Experiment 1: Ground Fescue Seed

The results of Exp. 1 are presented in Table 2. Total feed intake exhibited an endophyte*temperature interaction ($P = 0.001$) as feed intake was slightly lower with E+ at 22°C but greatly reduced with E+ at 32°C. Rate of intake tended to exhibit an endophyte*temperature interaction ($P = 0.064$) as rate of intake was greatest for E- at 22°C and similar for the other treatments. The number of meals consumed daily was reduced ($P = 0.001$) at 32°C. Respiration rate also exhibited an endophyte x temperature interaction ($P = 0.01$) as respiration rate increased at 32°C but the increase was much greater for E+. Core temperature exhibited an endophyte× temperature interaction ($P = 0.001$) as core temperature was elevated at 32°C but the increase was greater for E+. Skin temperature increased ($P = 0.001$) at 32°C, but unchanged by treatment ($P = 0.15$). Serum prolactin concentrations did not differ between treatments on d1 or d7 ($P = 0.82$ and 0.61, respectively); however, steers dosed with E+ had serum prolactin concentrations not different from zero ($P = 0.02$) on d7 while prolactin levels of steers dosed with E- were different from zero ($P = 0.16$; Figure 2A).

3.3.2) Experiment 2: Fescue Seed Extract

The results of Exp. 2 are presented in Table 3. Both E+ treatment and 32°C reduced total feed intake ($P < 0.05$), rate of intake ($P < 0.001$), and meals ($P < 0.001$), however, the interaction of endophyte treatment and temperature was not significant for any of these measures. In addition, respiration rate ($P = 0.003$), heart rate ($P = 0.044$), and core body temperature ($P = 0.001$) all had endophyte×temperature interactions. Respiration rate was increased by temperature but the increase was greatest for E+ at 32°C. Heart rate was decreased by E+ but the decrease was less for E+ at 32°C. Core temperature
was increased by E+ but the increase was greatest at 32°C. Systolic blood pressure was unaffected by treatment or ambient temperature, whereas, diastolic blood pressure tended \((P = 0.08)\) to increase with E+ treatment and tended \((P = 0.064)\) to decrease at 32°C. Dosing E+ extract tended to reduce serum prolactin concentrations on d7 as compared to E- dosing \((P = 0.10)\). Steers dosed with E+ had serum prolactin levels not different from zero on d7 \((P = 0.032, \text{Figure 2B})\).

3.4) Discussion

During seed dosing, animals received 4.1 mg\(\text{hd}^{-1}\text{d}^{-1}\) total ergovaline (ergovaline and ergovalinine), with extract dosing adjusted to provide a similar quantity. During both experiments alkaloid levels were lower than previously reported levels of 10.9 mg\(\text{hd}^{-1}\text{d}^{-1}\) (total ergovaline + ergovalinine; (Aiken et al., 2007). However, the observation of fescue toxicosis symptoms, including reduction of serum prolactin, indicates that alkaloid levels were sufficient in both experiments to induce toxicosis. After completion of the experiments, further analysis of the seed and extract indicated the presence of ergotamine (2.9 ppm in seed). The presence of ergotamine is not likely due to \textit{N. coenophialum}, but rather contamination by \textit{Claviceps purpurea}. Thus, animals received approximately 2.9 mg ergotamine daily as well.

A reduction in serum prolactin is considered the definitive indicator of fescue toxicosis in cattle. Dopamine D2 receptors are stimulated by ergot alkaloids (Dyer, 1993; Larson et al., 1994), which results in a reduction in prolactin secretion by the anterior pituitary. Reductions in both circulating and releasable levels of prolactin in cattle that had grazed endophyte infected tall fescue pastures have been reported (Schillo et al., 1988) and cultured pituitary cells treated with ergocryptine had reduced prolactin gene transcription (Maurer, 1981). Steers in this study had serum prolactin concentrations that were greatly decreased and not different from zero after 7d of E+ dosing for both seed and extract at 32°C. Aiken et al. (2007) reported that prolactin concentrations can be reduced as quickly as 4 h after feeding infected tall fescue seed. During the E-treatments, steers had serum prolactin concentrations averaging 93.4 and 212.7
ng/mL after 7 d on seed and extract treatments, respectively. The apparent reduction in prolactin concentrations of E- animals dosed with seed may be due to natural fluctuations of prolactin concentrations in cattle (Koprowski and Tucker, 1973; Tucker et al., 1974) as our values represent only a single daily sample. Serum prolactin concentrations in cattle (Stanisiewski et al., 1988; Dahl et al., 2000) and sheep (Jackson and Jansen, 1991) have been shown to vary with day length and temperature (Peters et al., 1981). Age of animals can also affect prolactin concentrations, with older animals having higher concentrations (McCarthy et al., 1979). However, as the temperatures in this experiment were at or above thermoneutral for cattle, they should not have altered prolactin secretion. Likewise, as all animals were of a similar age, this should not be a source of prolactin fluctuation. Animals in this study were given a minimum of 3 wks washout between E- and E+ treatments. Previous research indicates that serum prolactin concentrations increase and stabilize as quickly as 3 d after removal of infected tall fescue from the diet (Aiken et al., 2007). However, other studies have shown that prolactin concentrations can continue to increase for 28d after dietary change (Aiken and Piper, 1999). Collectively, these studies show that the variations in prolactin concentrations may be due to a number of factors, including individual animal variation in sensitivity to temperature and/or recovery time. However, it does not affect the value of prolactin as an indicator of fescue toxicosis as concentrations in steers receiving the E+ treatments at 32°C were dramatically reduced such that they were not different from zero.

It is well known that cattle exhibit changes in physiological responses at differing temperatures. In this experiment ambient temperature reduced total intake and meals during both seed and extract dosing. Rate of intake was reduced by temperature during extract dosing and tended to be lowered during seed dosing. It is well documented that high environmental temperatures negatively affect intake (Hahn, 1999). In addition, increased temperature resulted in an increase in respiration rate during both seed and extract dosing; this is generally attributed to efforts by the animal to decrease the thermal load by increasing respiratory cooling (Cisneros and Goins, 2009). Core temperature
was also increased at 32°C during all treatments; this is consistent with previous research indicating that core temperature is affected by environmental temperature (Johnson, 1965; Finch, 1986). Similarly, skin temperature was higher at 32°C during seed dosing. Elevation of ambient temperature increases the thermal load on animals and if cooling mechanisms are unable to compensate, core body and skin temperatures will be increased (Fuquay, 1981; Hahn, 1999).

Some research has indicated that reduced intake and heat stress may slightly depress heart rate (McGuire et al., 1989; Lough et al., 1990). Heart rate has been shown to be correlated with energy expenditure in cattle (Brosh et al., 1998b). In this experiment heart rate was decreased at 32°C during extract dosing and unaffected during seed dosing. This reduction in heart rate may be related to the reduced intake and available energy. These indicators show that the elevated temperatures in this experiment resulted in typical physiological responses. The temperature increase was used solely as a tool to evaluate efficacy of seed and extract dosing as the adverse effects of fescue toxicosis are exacerbated at higher ambient temperatures (Osborn et al., 1992; Aldrich et al., 1993a).

Animals consuming infected tall fescue under elevated environmental temperatures often exhibit a reduction in intake of 10 to 50% (Bond et al., 1984; Stuedemann and Hoveland, 1988). The animals in this experiment did not have reduced intake at 22°C, but had 28% and 19% reductions in intake resulting from dosing E+ seed and extract, respectively, at 32°C. Reductions in intake are not a certainty with endophyte consumption, even with elevated environmental temperatures. Heifers housed in temperature controlled rooms with daily cycling temperatures from 22°C to 32°C also showed no difference in intake between E+ and E- groups (Aldrich et al., 1993b). However, without diurnal temperature cycling an interaction of elevated temperature and E+ consumption is evidenced (Aldrich et al., 1993b). As the current experiment did not use daily temperature cycling, this interaction was present.
Ergot alkaloids act as agonists on serotonergic receptors (Dyer, 1993) which can depress intake, most likely through increasing satiety (Simansky, 1995b). Shorter grazing bouts have also been reported for animals consuming endophyte infected tall fescue (Dougherty et al., 1991). The number of meals per day was reduced by E+ dosing, and by elevated temperature for both seed and extract experiments. Likewise, rate of intake was reduced at 32°C and during E+ dosing for both seed and extract experiments of the current report. Other research has shown that passage rate decreases when animals consume endophyte infected tall fescue (Goetsch et al., 1987) which may also contribute to the reduced intake.

Respiration rate was elevated at 32°C for all treatments. Increased respiration serves as a method to dissipate heat (Hahn, 1999) and serves as an indicator of an animal’s thermal load (Gaughan et al., 2000). Therefore, the elevated respiration rate at 32°C observed in this experiment was expected. As seen in the present research, endophyte infected tall fescue has been shown to increase respiration rate of cattle above that caused by heat stress (Carr and Jacobson, 1969; Osborn et al., 1992). Lung tissue has α- and β-adrenergic receptors (Porcelli and Bergofsky, 1973) as well as serotonergic 5-HT receptors (Sadavongvivad, 1970). Stimulation of serotonergic receptors have been shown to have a bronchoconstrictive effect (Oliver, 1997). The alpha-2 adrenergic receptors present on blood platelets increase their production of thromboxane when stimulated by ergot alkaloids, which can cause platelet aggregation (Oliver, 1997). These combine to result in a reduction in blood $O_2$ saturation and tissue oxygenation in animals suffering from fescue toxicosis and lead to an increase in respiration rate in order to sufficiently oxygenate tissues (Oliver, 1997). The combination of both elevated environmental temperature and E+ treatment resulted in an increase in respiration over either temperature or treatment alone. This would indicate that the mechanism by which each of these alter respiration are separate and combinatory.

Increased blood pressure has been noted in some studies of ergot alkaloid effects. Research conducted using intravenous injection of alkaloids
tends to show this increase (Browning and Leite-Browning, 1997; Browning, 2000; McLeay et al., 2002) while studies that use ground E+ fescue seed or animals grazing high endophyte pastures do not report an increase in blood pressure (Rhodes et al., 1991; Aiken et al., 2007). Therefore, increased blood pressure may be an artifact of infusion and not directly caused by the ergot alkaloids as present in the infusates. It may require a secondary metabolite or greater concentrations of toxin than are routinely achieved with feeding.

Numerous studies have shown a reduction in heart rate with consumption of infected fescue (Osborn et al., 1992; Browning and Leite-Browning, 1997; Aiken et al., 2007). This reduction is often seen without the increase in blood pressure noted above. In the present experiment, reduced heart rate was seen during E+ dosing of both seed and extract. Blood pressure was only measured during extract dosing, and showed an increase in diastolic pressure due to E+. These results are in agreement with the theory that the heart rate of animals is not directly affected by the alkaloids, but rather is reduced in response to the elevated blood pressure caused by vasoconstriction, allowing for the maintenance of homeostasis (Oliver, 2005). The interaction of temperature and endophyte on heart rate is likely a combination of effects on blood pressure and core temperature. In hyperthermia, heart rate has been shown to increase by up to 25 beats per minute per degree Celsius in humans in order to facilitate increased cutaneous heat transfer (Desforges and Simon, 1993). Homeostatic maintenance of blood pressure overriding this increase in heart rate is the most probable explanation for the reduced heart rate at 32°C during E+ dosing in both experiments.

A number of studies have reported a relationship between infected fescue and increased core body temperature (Boling et al., 1989; Aldrich et al., 1993a; Al-Haidary et al., 2001). Similarly, increased core temperature is seen during heat stress situations (Finch, 1986; Lefcort and Adams, 1996; Eigenberg et al., 2005). In agreement with this, core temperature was elevated at the 32°C temperature as well as by E+ dosing during both seed and extract experiments. As discussed below, increased core temperature during E+ dosing is related to a
reduced cutaneous heat transfer. At times of elevated environmental temperature, cutaneous heat transfer becomes more important to maintain core temperature (Sessler et al., 1990). Animals consuming E+ seed in heat stress conditions are less able to dissipate heat at the time of greatest need. This results in the interaction of temperature and endophyte on core body temperature seen in the current experiments.

Increased core temperature is often in association with a decrease in skin temperature (Browning and Leite-Browning, 1997; Browning, 2000) that is attributed to the α-adrenergic receptor mediated vasoconstriction in subcutaneous areas (Dyer, 1993; Oliver, 1997) and the resulting reduced blood flow. Vasoconstriction in subcutaneous tissues has been postulated to result in a reduction of heat loss, thereby contributing to the increased core body temperature. However, this study showed no change in skin temperature over the ribs. Rhodes et al., (1991) reported that rib skin in steers had reduced blood flow during E+ treatment. Therefore, a corresponding reduction in temperature was expected in this experiment. It is possible that due to the temperature and humidity controlled environment and lack of radiant heat, the change in skin temperature was minimized and undetectable. Previous research has shown more pronounced changes in skin temperature for peripheral locations (Walls and Jacobson, 1970; Schmidt and Osborn, 1993b; Browning, 2004), as these locations were not measured in this experiment, it is possible that a change in skin temperature occurred and was not detected due to choice of measurement location.

3.5) Conclusion

Ruminal dosing of ground seed and seed extract were able to mimic the classic symptoms of fescue toxicosis in cattle. This model whereby seed or extract is directly dosed into the rumen eliminates the possibility of reduced alkaloid intake due to refusal of feed by the animal. This model may allow for more precise and repeatable dosing of alkaloids in future fescue research and may enhance the ability to study underlying mechanisms of fescue toxicosis.
Table 3.1: Chemical Composition of Basal Hay and Mineral Supplement

<table>
<thead>
<tr>
<th></th>
<th>Fescue Hay</th>
<th>Trace Mineral Premix</th>
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</thead>
<tbody>
<tr>
<td><strong>Fescue Hay</strong></td>
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<td></td>
</tr>
<tr>
<td>Dry Matter</td>
<td>85.8</td>
<td></td>
</tr>
<tr>
<td>CP, % DM</td>
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</tr>
<tr>
<td>NDF, % DM</td>
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</tr>
<tr>
<td>ADF, % DM</td>
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</tr>
<tr>
<td>Ash, % DM</td>
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</tr>
<tr>
<td>GE, kcal/g DM</td>
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</tr>
<tr>
<td><strong>Trace Mineral Premix</strong></td>
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<td></td>
</tr>
<tr>
<td>Zinc, min, ppm</td>
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<tr>
<td>Manganese, min, ppm</td>
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<td>Salt, min, %</td>
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<td>Iodine, min, ppm</td>
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<td>Cobalt, min, ppm</td>
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<td>Selenium, min, ppm</td>
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Table 3.2: Comparison of physiological measurements between steers ruminally dosed with ground fescue seed with ($S_{E^+}$) and without ($S_{E^-}$) endophyte at 22°C and 32°C

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment</th>
<th>$S_{E^-}$</th>
<th>$S_{E^+}$</th>
<th>SEM</th>
<th>$P = ^2$</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>22°C</td>
<td>32°C</td>
<td>22°C</td>
<td>32°C</td>
</tr>
<tr>
<td>Intake, kg/d</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rate of Intake, h$^{-1}$</td>
<td></td>
<td>1.44</td>
<td>1.07</td>
<td>1.02</td>
<td>1.06</td>
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<tr>
<td>Meals, d$^{-1}$</td>
<td></td>
<td>10.2</td>
<td>8.3</td>
<td>9.2</td>
<td>8.0</td>
</tr>
<tr>
<td>Respiration Rate, breaths/min</td>
<td></td>
<td>38.4</td>
<td>54.1</td>
<td>38.5</td>
<td>77.9</td>
</tr>
<tr>
<td>Heart Rate, beats/min</td>
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<td>81.1</td>
<td>84.1</td>
<td>72.3</td>
<td>71.6</td>
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<tr>
<td>Core Temperature, °C</td>
<td></td>
<td>37.5</td>
<td>38.1</td>
<td>37.9</td>
<td>38.2</td>
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<td>Skin Temperature, °C</td>
<td></td>
<td>35.1</td>
<td>36.4</td>
<td>35.4</td>
<td>36.8</td>
</tr>
</tbody>
</table>

$^1$ Data are presented as least squared means of animals dosed with $S_{E^+}$ and $S_{E^-}$ treatments (n = 4).

$^2$ Probability of a greater F statistic
Table 3.3: Comparison of Physiological Measurements between Steers dosed with Fescue Seed Extract With (E_{E+}) and Without (E_{E-}) Endophyte at 22°C and 32°C \(^1\)

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment</th>
<th>(P = ^2)</th>
<th>SEM</th>
<th>Main Effects</th>
<th>Interaction</th>
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<tr>
<td></td>
<td>(E_{E-})</td>
<td>(E_{E+})</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>22°C</td>
<td>32°C</td>
<td>22°C</td>
<td>32°C</td>
<td></td>
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<tr>
<td>Intake, kg/d</td>
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<td>8.29</td>
<td>8.50</td>
<td>6.70</td>
<td>0.644</td>
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<td>0.043</td>
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<td>0.021</td>
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<td>Rate of Intake, h(^{-1})</td>
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<td>1.36</td>
<td>1.08</td>
<td>0.195</td>
</tr>
<tr>
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<td></td>
<td></td>
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<td>0.005</td>
</tr>
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<td>Meals, d(^{-1})</td>
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<td>11.9</td>
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<td>&lt;0.001</td>
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<td>0.209</td>
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<td>Respiration Rate, breaths/min</td>
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<td>38.3</td>
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<tr>
<td>Heart Rate, beats/min</td>
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<td>&lt;0.001</td>
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<td>&lt;0.001</td>
</tr>
<tr>
<td>Skin Temperature, °C</td>
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<td>35.3</td>
<td>35.4</td>
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<td>Systolic Blood Pressure, mmHg</td>
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<td>0.110</td>
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<td>Diastolic Blood Pressure, mmHg</td>
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<td>54.8</td>
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\(^1\) Data are presented as least squared means of animals dosed with \(S_{E+}\) and \(S_{E-}\) treatments (\(n = 4\)).

\(^2\) Probability of a greater F statistic
Figure 3.1: Serum prolactin concentrations on d 1 and d 7 for steers dosed with endophyte free (E-) and endophyte infected (E+) tall fescue seed Exp. 1 (A) and fescue seed extract Exp. 2 (B). D1 venipunctures were performed prior to 0800 dosing at 22°C. D7 venipunctures were performed prior to 0800 dosing at 32°C. Environmental temperature was increased on D4. Fig 1A, D1 E+ v. E- P =0.84, D7 E+ v. E- P =0.64, E- D1 v. D7 P =0.39, E+ D1 v. D7 P =0.27, SEM = 122.19; Fig 1B, D1 E+ v. E- P =0.74, D7 E+ v. E- P =0.10, E- D1 v. D7 P =0.70, E+ D1 v. D7 P =0.09, SEM = 116.37.
CHAPTER 4: ALTERATION OF FASTING HEAT PRODUCTION DURING FESCUE TOXICOSIS IN HOLSTEIN STEERS

4.1) Introduction

The ergot alkaloids produced by this the fungal endophyte Neotyphodium coenophialum cause health and production issues when infected tall fescue is ingested by grazing animals, resulting in negative economic effects for producers (Hoveland, 2007). The decrease in productivity caused by fescue toxicosis has been estimated to cost United States livestock industry more than $1 billion per year (Strickland et al., 2011).

Animals consuming infected fescue exhibit a 10-50% reduction in intake and can have significant weight losses (Bond et al., 1984; Stuedemann and Hoveland, 1988). It is unlikely that this reduction in weight is due solely to reduced feed intake. Changes in organ mass, gene expression, and stress can affect energy metabolism, altering nutrient availability and use. Splanchnic tissues account for up to 25% of total body energy expenditure (McBride and Kelly, 1990) and 40-50% of oxygen uptake (Huntington, 1990). A reduction in intake can alter splanchnic energy use as a result of reduced visceral mass (Seal and Reynolds, 1993; McLeod and Baldwin, 2000). Whole body energy use may also be altered by alkaloid consumption. Iason and Murray (1996) found that sheep dosed with plant phenolic compounds had increased whole body energy use and increased urinary energy output. Several studies report that consumption of ergot alkaloids and reduced ME intake may interact to alter energy metabolism in rats consuming endophyte-infected tall fescue (Zhang et al., 2002; Spiers et al., 2005a). However, minimal research has been conducted to examine and separate the effects of reduced energy intake and alkaloid consumption.

The majority of research on fescue toxicosis has relied on animal consumption to introduce alkaloids into the system. This experiment used a ruminally dosed animal model to bypass the possibility of a reduction in intake altering the quantity of alkaloids ingested by the animal over the course of the
experiment. In addition, pair-feeding was utilized to separate the effects of reduced energy intake and alkaloid consumption on energy metabolism. The goal of this experiment was to use these methods to evaluate the interaction between consumption of endophyte-infected tall fescue and environmental temperature on basal metabolism in Holstein steers.

4.2) Materials and Methods

All procedures involving animals were approved by the University of Kentucky Animal Care and Use Committee.

Six Holsteins steers (BW = 348 ±13 kg), surgically fitted with ruminal cannulas (Bar Diamond Inc., Parma, ID), were weight matched into pairs for the duration of a two period cross-over design experiment. Each period consisted of two temperature segments, one each at 22°C and 30°C. Steers were housed in individual pens in temperature and humidity controlled rooms and fed once daily at 0730. Steers were previously adapted to a basal diet consisting of alfalfa cubes fed at 1.5 x NE_m, top dressed with 40 g trace mineralized salt (Table 1). Water was available *ad libitum* throughout the experiment.

During each segment, one steer per pair was ruminally dosed twice daily at 0730 and 1630 with 0.5 kg of ground endophyte-infected tall fescue seed (E+), the other animal in each pair received ground endophyte-free tall fescue seed (E-) for 7 d. Fescue seed from each seed lot was collected using a bag trier (#236, Seedboro, Chicago, IL). A minimum of ten bags were sampled within each lot. Quantitative determination of ergot alkaloids was performed using ultra performance liquid chromatography/tandem mass spectrometry using an Acquity UPLC®-TQD (Waters, Inc., Milford, MA) as described previously (Foote et al., 2012a). Briefly, 5 µL of the diluted sample was injected (full loop mode) onto an Acquity UPLC® BEH column (C18, 1.7 mm particle size, 2.1 x 100 mm; Waters, Inc.). Separation was accomplished with a linear binary gradient using water with 0.04% NH4OH (A) and acetonitrile with 0.04% NH4OH (B) and a constant flow of 0.50 mL per minute. Gradient program conditions were as follows: initial to 0.6 min – 100% eluent A; at 6.0 min – 10% eluent A/90% eluent B; at 6.1 to
8.5 min – 100% eluent B; at 8.6 to 10 min – 100% eluent A. Detection was accomplished by running the triple-quad mass detector (TQD; Waters, Inc.) in the MS-MS mode following positive electrospray ionization. Concentrations of each alkaloid were determined using a calibration curve with an internal standard (methysergide, 5.0 fmol on column). The calibration curves were linear ($R^2 > 0.97$) within a range of 5 to 250 fmol. Area under the curve values for both the “ine” and “inine” epimers were summed for quantitation as interconversion of the epimers readily occurs in solution.

Steers were offered alfalfa cubes at 1.5x NE\textsubscript{m} during E+ dosing. During E-dosing, intake was restricted to be equal to the intake of the assigned E+ pair on the corresponding day. Feed samples were collected daily by random grab and composited over the experiment. A portion of this sample was analyzed (Dairy One, Ithaca, NY, USA) for nutrient composition. Dry matter and CP were determined using methods 930.15 and 990.03 respectively (AOAC, 2005). Neutral detergent fiber and ADF were determined according to the methods of Van Soest et al. (1991) using filter bags in an AnkomA200 Digestion Unit (Ankom Technology, Macedon, NY). Net energy was calculated using tabular values (NRC, 2001) and net energy for maintenance was calculated using equations from NRC (2001).

At 0700 on d 8 of each segment, animals were moved to individual metabolism stalls fitted with indirect calorimetry head-boxes as previously described by Koontz et al. (2010). In order to minimize the time between feeding and fasting heat production (FHP) determination, ruminal contents were evacuated by suction. This method provides an alternative to the traditional 48 to 72 h fasting prior to FHP measurement. After removal, ruminal contents were weighed and subsampled for DM determination, covered with hay to minimize drying, and maintained at 39\textdegree C in a forced air oven. Ruminal content DM was determined by drying in a forced air oven at 60\textdegree C to a constant weight. Following evacuation, the reticulorumen was rinsed with warm (39\textdegree C) physiological saline, emptied, and filled with a buffer solution (Table 2) at 39\textdegree C, that was gassed with 75\% N\textsubscript{2} ad 25\% CO\textsubscript{2} prior to rumen incubation (Kristensen and Harmon, 2004).
After buffer introduction animals were fasted for 12-h prior to data collection. A 12-h fast is sufficient to bring the respiratory quotient of cattle to a stable baseline level following rumen evacuation (Kim et al., 2013). During buffer incubation and FHP determination E+ or E- fescue seed extract (Koontz et al., 2012a) was added at 12 h intervals to maintain treatment presentation to the animal. The E+ extract provided quantities of ergovaline + ergovalinine that were similar to the E+ seed whereas the quantity of E- extract was similar to E+.

The collection period consisted of a 16-h determination of heat production via indirect calorimetry. Inspired and expired air was analyzed for O2 and CO2 concentrations at 12-min intervals using Oxymax software (Columbus Instruments, Columbus, OH). Air flow was measured by individual mass flow meters and maintained at 600 L/min. In addition, heart rate was continually measured using a telemetry device attached to a heart-girth band (WearLink, Polar Brand, Brooklyn, NY) during fasting heat production determinations.

Urine was collected during the 16-h FHP determination via continuous suction using a rubber funnel system attached to the ventral portion of the abdomen which allowed collection of urine into a plastic collection vessel. Urine acidity was reduced to pH < 3 by adding a 23.5% solution of H3PO4 to the collection vessel to prevent ammonia N loss. Urine output weight was recorded and subsampled for each period and steer. Samples were stored at 0°C until analysis. Nitrogen content of wet urine as determined on an Elementar varioMAX (Elementar Analysensysteme, Hanau, Germany).

Whole-body heat production over the 16 h collection period was calculated by indirect calorimetry using a modification of the equation proposed by Brouwer (1965) as follows:

\[
HP \ (\text{kcal}) = 3.869 \ (L_{O2}) + 1.195 \ (L_{CO2}) - 1.431 \ (g_{UN})
\]

Where HP is heat production, \( L_{O2} \) is oxygen consumed (L), \( L_{CO2} \) is carbon dioxide produced (L), and \( g_{UN} \) is urinary nitrogen excretion (g). Methane was not included in the equation as measured methane production was negligible during the 16h measurement period for all animals.
After FHP determination, ruminal contents were replaced and animals returned to individual pens and fed their basal diets for 7 d then room temperature was increased to 30°C and the dosing, fasting, and measurement procedures were repeated.

Following the second FHP determination, animals were returned to individual pens for a 14 d washout period where they were maintained on the alfalfa cubes at 1.5 X NEm prior to the cross-over period. The cross-over period was conducted on the same time table (7 d fescue seed dosing prior to starting measurements); however, the environmental temperatures were presented in reverse order to the first (30°C followed by 22°C), with each steer within a pair on the alternate endophyte treatment.

4.2.1) Statistical Analysis

The data were analyzed as a crossover experiment with a 2x2 factorial treatment structure, using the Mixed procedure of SAS (SAS Institute, Cary, NC) with individual steer as the experimental unit. Animal and period were considered random effects, while endophyte treatment and environmental temperature were fixed effects. Data were analyzed for effects of treatment, temperature and the interaction of treatment×temperature. All results are presented as least squared means of all data collected within each temperature phase. Indirect calorimetry data (O₂ consumption, CO₂ production, and respiratory quotient) and heart rate were averaged across the 16h measurement period prior to analysis. Sums of squares were partitioned into the main effects of endophyte and temperature as well as their interaction. Data are presented as least squares means ± SEM. Treatment effects were considered significant at \( P \leq 0.05 \).

4.3) Results and Discussion

Both seed and extract dosing provided 4.1 mg•hd⁻¹•d⁻¹ total ergovaline (ergovaline plus ergovalinine). This level, as well as the 7-d ruminal dosing
method, were previously shown to be sufficient to induce fescue toxicosis (Koontz et al., 2012a).

Historically the most severe effects of fescue toxicosis are seen in the summer, (Sessler et al., 1990; Thompson and Stuedemann, 1993) this is believed to be due to contradiction of natural vasorelaxation necessary for thermoregulation at elevated ambient temperatures by ergot alkaloid induced vasoconstriction (Gadberry et al., 2003). Vasoconstriction reduces the ability of the animal to dissipate heat, leading to behavioral alterations such as increased time spent standing in shade and reduced time eating (Bond et al., 1984). Both heat stress and altered plane of nutrition have significant affects on metabolic activity individually (Birkelo et al., 1991; O'Brien et al., 2010). Heat stress and reduced level of intake have discrete effects on milk production and hormone levels in dairy cattle (Rhoads et al., 2009), and growth rate and hormone concentrations of growing bull calves (O'Brien et al., 2010), indicating that separating the effects of heat and reduced intake are crucial to understanding the metabolic changes that occur during fescue toxicosis. However, in this experiment, no interaction between treatment and temperature was observed \( P > 0.10 \). Additionally, in the present study, increased environmental temperature had no effect except to reduce DM intake by 17% \( P = 0.004 \). Animals were offered feed at 1.5 x NEm. At 22°C consumption equaled 1.22 x NEm, while at 30°C consumption was 1.03 x NEm. There was no difference in intake between endophyte treatments \( P = 0.931 \) due to the pair feeding design. This lack of intake difference and effect of elevated environmental temperature indicates that observed differences can be attributed to the alkaloids present in endophyte-infected tall-fescue.

Treatment with endophyte infected seed increased the DM percentage \( P < 0.0001 \) and total DM weight \( P < 0.0001 \) of ruminal contents, while total weight of ruminal contents was not different between treatments \( P = 0.149 \). Considering that the animals in this study were pair fed to remove intake differences, these data suggest that there is alteration of rumen kinetics, possibly resulting in a reduction in particulate passage from the rumen of animals.
consuming endophyte infected tall fescue. α-Adrenergic and serotonergic receptors have been implicated in control of gastrointestinal tract motility (Talley, 1992; van Miert et al., 1994); both of these receptor types are acted on by alkaloids present in endophyte-infected tall fescue (Larson et al., 1999; Schoning et al., 2001). Hannah et al. (1990) reported increased ruminal liquid outflow rates and decreased OM intake in sheep fed a diet consisting primarily of fescue seed and soybean hulls when E+ seed was used to provide 1.5 or 3 ppm ergovaline as compared to E- seed. Conversely, Goetsch et al. (1987) observed decreased liquid and particulate passage rates with decreasing intake of five diets containing increasing percentages of endophyte-infected hay (range from 0 to 1612 ppm N-acetyl + N-foryl loline) included in the diet of dairy steers, but no effect on liquid passage rate. Whereas Forcherio et al. (1995) observed no difference in ruminal passage rate between steers consuming E+ and E- tall fescue hay, despite reduced intake with E+ hay. These differing results may be due to differences in treatment structure, diet, or environmental factors, all of which are known to affect ruminal passage rates. Studies comparing intake of E+ and E- feedstuffs are often confounded by feedstuff. Further research controlling these factors is needed to accurately determine if ruminal passage rates are altered by ergot alkaloid ingestion.

Heart rate was unaffected by endophyte treatment ($P = 0.953$) or temperature ($P = 0.555$). This contradicts previous research where E+ fescue consumption resulted in reduced heart rates (Osborn et al., 1992; Aiken et al., 2007). Heart rates recorded in this study were lower than previous fescue studies (Browning and Leite-Browning, 1997; Koontz et al., 2012a). As this observation was seen in both endophyte treatments and at both 22°C and 30°C, it is most likely an effect of the methodology of the current experiment. Bradycardia has been previously shown to occur during fasting and following rumen evacuation (Clabough and Swanson, 1989; McGuirk et al., 1990). This is believed to be caused by increased parasympathetic activity mediated by the vagal nerve, as a result of reduced reticulo-rumen tension (Clabough and Swanson, 1989). Thus, the lack of treatment effect on heart rate in the present
study is likely due to heart rate being at a physiological minimum due to rumen evacuation and fasting, with no opportunity available for further depression by ergot alkaloid ingestion.

Urinary output, as well as %N of the urine, were not affected by endophyte treatment or environmental temperature. This is in agreement with previous research reporting no difference in urinary N excretion in *ad libitum* fed steers (Matthews et al., 2005) and pair-fed (manual insertion via cannula) lambs (Fiorito et al., 1991) consuming E+ and E- hay. This may indicate that ergot alkaloid ingestion does not appreciably alter N metabolism. In the present study, oxygen consumption was reduced (*P = 0.040*) in E+ dosed animals, while carbon dioxide production tended to be reduced (*P = 0.070*). These changes led to a lower average RQ for E- animals as compared to E+ (0.723 and 0.738 respectively; *P = 0.022*). In addition, calculated FHP was lower (*P = 0.006*) for animals dosed with E+ fescue seed. Aldrich et al. (1993a) also observed a reduction in heat production in lambs consuming a diet containing endophyte-infected fescue at 1.5% of BW, with a high ergovaline intake (0.118 mg/kg BW.75) at 32°C. In contrast, no difference in heat production was found between endophyte treatments in steers fed *ad libitum* receiving slightly higher levels of ergovaline (0.021 mg/kg BW.75 vs. 0.012 mg/kg BW.75) than the present experiment (Aldrich et al., 1993b). As heat production and intake are closely linked (Church, 1988), it is possible that differences in heat of fermentation, digestion, and other processes are responsible for the discrepancy in results between previous experiments and the current determination of fasting heat production.

Also in agreement with the present experiment, rats fed *ad libitum* and injected with ergovaline showed acute reductions in metabolic rate that were greater at thermoneutral temperatures than during either heat or cold stress (Spiers et al., 1995; Zhang et al., 2002). The energy cost of thermoregulatory activities of the animals at high and low environmental temperatures (i.e. shivering or panting) may explain the increased metabolic rate at temperatures outside the thermoneutral range (Stanier et al., 1984). However, in the present
study no difference was observed in FHP at differing ambient temperatures. The greatest alterations in body temperature related to fescue toxicosis occur during temperature transitions in both cattle (Scharf et al., 2010) and rats (Settivari et al., 2008a). It is possible that as animals in this study were maintained at 30°C for 7d prior to FHP determination, that no additional energy was required to maintain body temperature. Similarly, Aldritch et al (1993b) saw no difference in heat production between steers on E+ and E- treatments at 32°C.

With a protocol that involves dosing into a blank ruminal buffer, there is also a possibility that the results seen are an acute effect of extract dosing, rather than from the chronic seed dosing. However, examination of oxygen consumption over the 16h measurement period shows no change in O₂ consumption following extract dosing (Figure 1). Further, the slopes of all four treatments are not different from zero (P>0.1). Thus, the treatment differences in FHP seen in this experiment are due to ingestion of fescue seed over the 7d period, not due to acute effects of extract dosing during FHP determination.

Possible mechanisms underlying a chronic reduction in FHP during fescue toxicosis in cattle are reduced service organ size and alterations in gene expression, resulting in changes in energy use. While neither of these was measured in the present experiment, both have been shown to occur in previous research regarding fescue toxicosis. As maintenance energy can be defined as the sum of FHP and energy used for digestion, the use of rumen evacuation methodology for determination of fasting heat production may provide a more accurate indication of maintenance energy requirements than the traditional 48 to 72 h fast. Rumen evacuation allows for a minimum of time between rumen emptying and data collection, likely reducing alteration in energy usage by gastrointestinal tissues that occur because of fasting.

Brown et al. (2009) reported that liver weights were greater for steers grazing low-endophyte pastures as compared to animals on pastures with high-endophyte infection levels; though no differences were seen in heart or kidney weights. However, as animals were on pasture, intakes were not measured by Brown et al. (2009), thus it cannot be determined if these reported differences
are due to level of intake or alkaloid ingestion. Similar results have also been reported for rats with the same confounding of intake and endophyte treatment (Chestnut et al., 1992; Settivari et al., 2006; Settivari et al., 2008a). As much as 25% of whole-body energy use can be attributed to the hepatic tissues (Eisemann and Nienaber, 1990), thus a decrease in organ size would represent a reduction in maintenance energy requirements (Reynolds, 2002). Liver weight is correlated to energy availability in intake restricted steers (Sainz and Bentley, 1997). In the present study energy intake was equal between E+ and E- treatments, thus liver size may not have been altered. However, using animals with equal intakes, Harmon et al. (1991) reported that energy use by splanchnic tissues was lower for steers consuming endophyte-infected tall fescue as compared to uninfected fescue. As organ mass is highly correlated to energy use (McLeod and Baldwin, 2000), a reduction in energy consumption by these tissues could be indicative of decreased organ size, despite equivalent energy intakes.

Consumption of endophyte infected tall fescue down regulates genes involved in metabolism in both hepatic (Settivari et al., 2006; Brown et al., 2009) and luteal tissues (Jones et al., 2004b). It is possible that the reduction in the activity of these genes is sufficient to reduce basal energy use as seen in the present experiment. In addition, a reduction in metabolic rate due to decreased oxidative phosphorylation would result in diminished ability to compensate for cold stress by increasing heat production through mitochondrial proton leak (Harper et al., 2002). This intolerance of cold temperatures and corresponding reduction in metabolic rate has been reported in rats fed E+ seed and subjected to cold temperatures (Spiers et al., 1995).

Several studies have shown that cattle entering the feedlot after grazing endophyte-infected tall fescue pasture exhibit compensatory gain and greater feed efficiency (Cole et al., 1987; Piper et al., 1987; Coffey et al., 1990; Lusby et al., 1990). The data presented here may provide a cause for this observation, though research evaluating the length of carryover effects after removal from endophyte-infected fescue is needed to confirm this theory. If consumption of E+
fescue causes a reduction in maintenance energy requirements, the animals would then be able to utilize the high levels of energy in feedlot diets more efficiently. Steers subjected to intake restriction during the growing phase, then re-fed, have been shown to have decreased maintenance requirements (Sainz and Oltjen, 1994). After cessation of alkaloid intake and adaptation to a concentrate diet, animals no longer exhibit this increased efficiency and growth. Cole (1987) and Lusby (1990) found that steers from E- and E+ pastures had the largest differences in gain during the first 7-weeks on feed and concluded that the improved feed efficiency of animals from endophyte infected pastures was not a permanent effect.

4.4) Conclusion

Ingestion of endophyte-infected tall fescue results in decreased fasting heat production in cattle. This is indicative of a reduction in maintenance energy requirements and may be related to a decrease in liver size or other metabolic activity. In addition, a reduction in metabolic rate may contribute to the compensatory gain often observed in cattle entering the feedlot after grazing endophyte-infected pastures.
Table 4.1: Chemical Composition of Basal Diet and Mineral Supplement

<table>
<thead>
<tr>
<th>Alfalfa Cubes</th>
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<tbody>
<tr>
<td>Dry Matter, %</td>
<td>85.8</td>
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<tr>
<td>CP, % DM</td>
<td>16.5</td>
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<tr>
<td>NDF, % DM</td>
<td>51.9</td>
</tr>
<tr>
<td>ADF, % DM</td>
<td>37.2</td>
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<tr>
<td>NEₘ, Mcal/kg DM</td>
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<table>
<thead>
<tr>
<th>Trace Mineral Salt Premix</th>
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<tbody>
<tr>
<td>Zinc, min, ppm</td>
<td>5500</td>
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<td>Manganese, min, ppm</td>
<td>4790</td>
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<tr>
<td>Salt, min, %</td>
<td>92</td>
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<tr>
<td>Salt, max, %</td>
<td>96</td>
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<tr>
<td>Copper, min, ppm</td>
<td>1835</td>
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<tr>
<td>Iron, min, ppm</td>
<td>9275</td>
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<tr>
<td>Iodine, min, ppm</td>
<td>115</td>
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<tr>
<td>Cobalt, min, ppm</td>
<td>65</td>
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<tr>
<td>Selenium, min, ppm</td>
<td>18</td>
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Table 4.2: Composition of Ruminal Buffer Solution

<table>
<thead>
<tr>
<th>Item</th>
<th>mmol/kg</th>
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<tbody>
<tr>
<td>NaCl</td>
<td>96</td>
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<tr>
<td>NaHCO₃</td>
<td>24</td>
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<tr>
<td>KHCO₃</td>
<td>30</td>
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<tr>
<td>K₂HPO₄</td>
<td>2</td>
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<tr>
<td>CaCl₂</td>
<td>1.5</td>
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<tr>
<td>MgCl₂</td>
<td>1.5</td>
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</tbody>
</table>
Table 4.3: Comparison of physiological measures and gas production between steers dosed with endophyte free (E-) and endophyte infected (E+) tall fescue seed at 22°C and 30°C<sup>1</sup>

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment</th>
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<tbody>
<tr>
<td></td>
<td>E- 22°C</td>
<td>348</td>
<td>349</td>
<td>348</td>
<td>346</td>
<td>13.4</td>
<td>0.675</td>
<td>0.955</td>
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<td></td>
<td>E+ 22°C</td>
<td>348</td>
<td>349</td>
<td>348</td>
<td>346</td>
<td>13.4</td>
<td>0.675</td>
<td>0.955</td>
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<tr>
<td></td>
<td>E- 30°C</td>
<td>346</td>
<td>349</td>
<td>348</td>
<td>346</td>
<td>13.4</td>
<td>0.675</td>
<td>0.955</td>
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<tr>
<td></td>
<td>E+ 30°C</td>
<td>346</td>
<td>349</td>
<td>348</td>
<td>346</td>
<td>13.4</td>
<td>0.675</td>
<td>0.955</td>
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<tr>
<td>SEM</td>
<td></td>
<td>13.4</td>
<td>0.931</td>
<td>0.955</td>
<td>0.691</td>
<td>0.691</td>
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<td>Body Weight (kg)</td>
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<tr>
<td>Intake (kg DM/kg BW&lt;sup&gt;75&lt;/sup&gt;)</td>
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<td>Ruminal contents (g/kg BW&lt;sup&gt;75&lt;/sup&gt;)</td>
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<td>Heart Rate (beats/min)</td>
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<td>O&lt;sub&gt;2&lt;/sub&gt; Consumption&lt;sup&gt;3&lt;/sup&gt; (L/kg BW&lt;sup&gt;75&lt;/sup&gt;)</td>
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<tr>
<td>CO&lt;sub&gt;2&lt;/sub&gt; Production&lt;sup&gt;3&lt;/sup&gt; (L/kg BW&lt;sup&gt;75&lt;/sup&gt;)</td>
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<tr>
<td>Urinary Output&lt;sup&gt;3&lt;/sup&gt; (g/ kg BW&lt;sup&gt;75&lt;/sup&gt;)</td>
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<td>Urinary Nitrogen (%)</td>
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<td>Respiratory Quotient</td>
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<tr>
<td>Heat Production&lt;sup&gt;3&lt;/sup&gt; (kcal/kg BW&lt;sup&gt;75&lt;/sup&gt;)</td>
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<sup>1</sup> Data are presented as least squares means of animals dosed with E+ and E- treatments at each temperature (n = 6).

<sup>2</sup> Probability of a greater F statistic

<sup>3</sup> Data presented as total production or consumption for the 16h measurement period.
Figure 4.1. Oxygen consumption during 16-h fasting heat production determination of steers dosed with endophyte infected (E+) or uninfected (E-) tall fescue dosed and housed at either 22°C or 30°C. Animals were dosed with ground seed at 0 and 12h. Slopes of lines for individual treatments are not significantly different from zero (E+TN $P = 0.153$, E+HS $P = 0.233$, E-TN $P = 0.104$, E-HS $P = 0.161$).
5.1) Introduction

Animals consuming endophyte-infected tall fescue exhibit reductions in intake and ADG (Bond et al., 1984; Stuedemann and Hoveland, 1988). A reduction in intake can alter splanchnic energy use as a result of reduced visceral mass, reducing energy requirements (Seal and Reynolds, 1993; McLeod and Baldwin, 2000). Ingestion of infected seed was previously shown to reduce fasting heat production (Koontz et al., 2012b). Further, ergot alkaloids can reduce blood flow to and nutrient absorption from the rumen epithelium (Foote et al., 2012b). Changes in organ mass, absorption, and intake can each affect metabolism, however, minimal research has been conducted to separate the effects of reduced energy intake and alkaloid consumption during fescue toxicosis.

Ruminal motility affects feed digestion and passage rate altering nutrient availability to the animal (Church, 1988). Both α-adrenergic and serotonergic receptors have been implicated in control of gastrointestinal tract (GIT) motility (Talley, 1992; van Miert et al., 1994); both of these receptor types are acted on by alkaloids present in endophyte infected tall fescue (Oliver et al., 1993; Schoning et al., 2001). Increased (Hannah et al., 1990), decreased (Goetsch et al., 1987), and unchanged (Forcherio et al., 1995) passage rates have been previously reported. However, experiments have not determined the changes in passage caused by alkaloid consumption while holding feed intake constant. Previous research has also shown increased (Schmidt et al., 1982; Goetsch et al., 1987), decreased (Westendorf et al., 1993; Aldrich et al., 1993b; Matthews et al., 2005), and unchanged (Harmon et al., 1991; Forcherio et al., 1995) digestibility related to ingestion of endophyte infected tall fescue. However, these studies compared the digestibility of tall fescue by comparing infected and uninfected forages or seeds. There is a lack of research examining the digestibility of a common feedstuff in animals consuming E+ and E- diets.
The majority of research on fescue toxicosis has relied on animal consumption to introduce alkaloids into the system. This experiment used a ruminally dosed animal model (Koontz et al., 2012a) to remove the possibility of a reduction in intake altering the quantity of alkaloids ingested by the animal over the course of the experiment. In addition, pair-feeding was utilized to separate the effects of reduced nutrient intake and alkaloid ingestion. The goal of this experiment was to use these methods to evaluate the interaction between consumption of endophyte-infected tall fescue and dietary intake level on whole body N and energy balance, diet digestion, and ruminal kinetics.

5.2) Materials and Methods

All procedures involving animals were approved by the University of Kentucky Animal Care and Use Committee.

5.2.1) Animals

Six Holsteins steers (BW = 217 ±7 kg), surgically fitted with ruminal cannulas (Bar Diamond Inc., Parma, ID), were weight matched into pairs for the duration of a cross-over design experiment with a 2x2 factorial treatment structure. Factors were endophyte (infected, E+ vs. uninfected, E-) and targeted feeding levels of 1.1 x NEm, (H) and 1.8 x NEm, (L; achieved 1.09 and 1.74 respectively). Steers were housed in individual pens in temperature and humidity controlled rooms and fed twice daily at 0700 and 1500. The basal diet consisted of alfalfa cubes fed at 1.5 x NEm, top dressed with 30 g trace mineralized salt (Table 1). Water was available ad libitum throughout the experiment.

After an 8 d intake level adaptation in each period, one steer per pair was ruminally dosed twice daily at 0730 and 1530 with ground endophyte-infected tall fescue seed (E+) to provide 0.0255 mg ergovaline + ergovalinine/kg BW• d⁻¹, the other animal in each pair received an equivalent amount of ground endophyte-free tall fescue seed (E-) for the duration of the 23 d period. Steers assigned to E+ treatment were offered alfalfa cubes at 1.1 or 1.8 x NEm. During E- treatment, intake was restricted to be equal to the intake of the assigned E+ pair on the
corresponding day. Feed and fescue seed samples were collected daily by random grab and composited over the experiment.

5.2.2) In Situ DM Disappearance

On d 13 to 16, Dacron bags (10cm x 20cm; pore size = 53 μm; Ankom, Fairport, NY) containing 5g ground (2mm screen, Model 4 Wiley Mill; Thomas Scientific, Swedesboro, NJ) alfalfa cubes were placed into the rumen of each animal. Bags were inserted in a weighted mesh bag in the rumen at appropriate intervals to allow for duplicate 2, 6, 12, 24, 48, and 72 h time points to be removed together at 72 h. Two blank Dacron bags incubated for 72 h were used to correct for microbial and feed contamination. Dry matter loss during rinsing was determined using two non-incubated bags for each animal. Upon removal, bags were rinsed five times in cold water (1min/rinse) using a commercial washing machine set at low water level, then dried at 55°C for 24 h, and reweighed to determine DM disappearance.

5.2.3) Nitrogen and Energy Balance

On d 17 to 21 of each period, animals were housed in individual metabolism stalls for complete fecal and urine collections. Feces and urine were collected at 0700 daily. Continuous suction using a rubber funnel system attached to the ventral portion of the abdomen allowed collection of urine into a plastic collection vessel. Urine acidity was reduced to pH < 3 by adding 1 L of a 23.5% solution of H₃PO₄ to the collection vessel in order to prevent ammonia N loss. Urine output weight was recorded daily. A constant percentage was subsampled daily to contribute approximately 250 g to a composite for each period and steer. This composite was stored at 0°C until analysis. The wet weight of fecal output was recorded daily for each steer. A subsample was taken at a constant percentage to give approximately 2 kg wet matter per day. Daily fecal samples were frozen at 0°C until the end of the experiment, when composited by period and steer using a Hobart mixer (Model H-600, Hobart Manufacturing Co, Troy, OH). The composite samples were stored at 0°C for
until analysis. Additional fecal samples were dried daily in a 100°C forced air oven to determine daily fecal DM output (AOAC, 2005).

During ruminal cannulation, a radio telemetry device (CorTemp, HQ Inc, Palmetto, FL) was placed in the abdomen of each steer which continually measured and recorded core temperature. Heart rate was continuously measured using a telemetry device attached to a heart-girth band (WearLink, Polar Brand, Brooklyn, NY). During d 17 to 19 core temperature and heart rate were measured at 1-min intervals.

On the final 2 d of excreta collection (d 20 to 21) the steers were placed in head-boxes for indirect calorimetry data collection, as previously described by Koontz et al. (2010). Measures of inspired and expired O₂, CO₂, and CH₄ were collected at 21-min intervals using Oxymax software (Columbus Instruments, Columbus, OH). Data were corrected O₂ and CO₂ recoveries of 105.3% and 102.8% respectively, as determined prior to conducting the experiment.

5.2.4) Ruminal Passage and VFA

On d 22 steers were intraruminally pulse dosed with 350 mL Cr-EDTA at 0700 to evaluate ruminal liquid passage rate (Udén et al., 1980). The Cr marker was administered throughout the rumen by injection via a stainless steel probe with a perforated tip. Ruminal fluid was collected by suction at 0 (before feeding, after Cr-EDTA dosing), 3, 6, 9, 12, 18, and 24 h post-dosing. At each time point 25 mL of rumen fluid was collected from the ventral rumen and pH immediately measured using a Model IQ150 probe (IQ Scientific Instruments, Carlsbad, CA). Five mL of fluid was then combined with 0.5 mL internal standard followed by 0.5 mL 50% (w/v) meta-phosphoric acid before freezing at -20°C (Erwin et al., 1961), until analysis. An additional 10mL of rumen fluid was stored at -20°C for later analysis.

On d 23 treatment effects on ruminal DM and indigestible ADF (iADF) were evaluated. Indigestible ADF was used as an indicator of particulate passage rate. Reticulo-rumen contents were evacuated by hand, weighed, mixed, and subsampled in triplicate prior to feeding (0700). Rumen contents
were replaced in the rumen and the animals were fed and dosed as normal. Four hours following feeding (1100) the evacuation procedure was repeated. Each subsample was dried to a constant weight in a 55°C forced air oven and stored in individual containers.

5.2.5) Chemical Analysis

Composite feed and fecal samples were dried at 55°C in a forced-air oven then ground through a 1 mm screen in a Wiley mill (Model 4; Thomas Scientific, Swedesboro, NJ). The dried ground samples were analyzed for dry matter and CP using methods 930.15 and 990.03 respectively (AOAC, 2005). Neutral detergent fiber and ADF were determined according to the methods of Van Soest et al. (1991) using filter bags in an Ankom A200 Digestion Unit (Ankom Technology, Macedon, NY). Feed net energy was calculated using tabular values (NRC, 2001) and net energy for maintenance was calculated using equations from NRC (2001). Heat of combustion was determined for feed, feces, and urine (Parr 1281 Bomb Calorimeter, Moline, IL). To measure urine GE, samples were oven-dried for 2 d at 55°C in polyethylene bags (Jeb Plastics Inc., Wilmington, DE) before combustion (Wilkerson et al., 1997). To obtain the urinary energy contents, the known heat of combustion per gram of bag material was subtracted from the total heat measured. Nitrogen content of dried feed and feces and wet urine were determined using an Elementar varioMAX (Elementar Analysensysteme, Hanau, Germany).

Rumen fluid samples were centrifuged at 39,000 x g for 20 min and the supernatant retained for analysis. Ruminal VFA concentrations were determined by gas chromatography (HP6890, Hewlett-Packard, Palo Alto, CA) using a 25326 Nukol® fused silica column (15m x 0.53mm x 0.5 um film thickness; Sigma/Supelco, Belfontaine, PA). Chromium concentration in the fluid was determined by atomic absorption spectrometer (PerkinElmer AAnalyst 200, PerkinElmer, Waltham, MA).

Dried rumen contents samples were composited by animal and period, then ground through a 1 mm screen in a Wiley mill (Model 4; Thomas Scientific,
Swedesboro, NJ) and analyzed for iADF by *in situ* incubation following the procedures of Vanzant et al. (2002). Triplicate Dacron bags (10cm x 20cm; pore size=53 μm; Ankom, Fairport, NY) containing 5g ground sample were prepared for each animal x time x treatment combination as well as feed, E+ seed, and E- seed samples. One replicate of each sample was placed into each of 3 weighted mesh bags. Each set of samples was then placed into the rumen of one of three animals grazing endophyte free fescue pasture for 168h. Upon removal, bags were rinsed as described above then dried at 55°C for 24 h, and reweighed to determine DM disappearance. Each Dacron bag was opened and 0.5g of the sample was transferred to an F57 filter bag (ANKOM Technology Corporation, Fairport, NY). The detergent analysis system (Goering and Van Soest, 1970) modified for the ANKOM 200 fiber unit (ANKOM Technology Corporation, Fairport, NY) was used to determine ADF for each sample.

5.2.6) Calculations

Dry matter disappearance was determined for each *in situ* replicate. The data were plotted as percent disappearance over time and fit to a one phase association model adapted from Ørskov and McDonald (1979) using GraphPad Prizm (GraphPad Software, San Diego, CA):

\[ Y = b \left( 1 - e^{-kt} \right) \]

Where Y is DM disappearance (%), b is DM fraction available over time, and k is fractional rate of disappearance.

Whole-body HP was calculated using the Brouwer (1965) equation as follows:

\[ \text{HP (kcal)} = 3.869 \left( \text{LO}_2 \right) + 1.195 \left( \text{LCO}_2 \right) - 0.516 \left( \text{LCH}_4 \right) - 1.431 \left( \text{gUN} \right) \]

Where HP is heat production, LO2 is oxygen consumed (L), LCO2 is carbon dioxide produced (L), LCH4 is methane produced (L), and gUN is urinary nitrogen excretion (g).

Partial efficiency was calculated as the slope of the line between the ratio of RE to ME at each intake, for each animal during each endophyte treatment.
Ruminal liquid volume and liquid dilution rate were estimated by linear regression of the natural logarithm of Cr concentration against sampling time as described by Warner and Stacy (1968) using GraphPad Prizm (GraphPad Software, San Diego, CA). Mean turnover time was calculated as the reciprocal of the fractional dilution rate. Rate of fluid outflow was calculated as:

\[ P = \frac{C_{rd}}{C_{rT0}} (FDR) \]

Where \( P \) is liquid passage rate (L/h), \( C_{rd} \) is concentration of chromium dose (mg), \( C_{rT0} \) is concentration of chromium at time zero (mg/L), and FDR is fractional dilution rate of chromium (%/h).

Particulate passage rate was calculated for each time point (pre-feeding and 4 h post-feeding) by dividing the rate of iADF intake (daily intake/24h) by the amount of iADF in the rumen at each time (Waldo et al., 1972). The average of these two time points is reported.

5.2.7) Statistical Analysis

The data were analyzed as a cross-over experiment with a 2x2 factorial treatment structure, using the Mixed procedure of SAS (SAS Institute, Cary, NC) with individual steer as the experimental unit. Animal and period were considered random effects, while endophyte treatment and intake level were fixed effects. Data were analyzed for effects of treatment, intake level and the interaction of treatment × intake. Sums of squares were partitioned into the main effects of endophyte and intake as well as their interaction. Data are presented as least squares means ± SEM. Treatment effects were considered significant at \( P \leq 0.05 \).

Indirect calorimetry data (O₂ consumption, CO₂ production, and respiratory quotient), heart rate, and core temperature were averaged across the measurement period prior to analysis. Ruminal VFA and pH data were analyzed as repeated measures in time.
5.3) Results and Discussion
5.3.1) Intake and Physiological Effects

Fescue seed dosing provided 0.0255mg total ergovaline (ergovaline + ergovalinine)/kg BW•d. This level, as well as the ruminal dosing method, were previously shown to be sufficient to induce fescue toxicosis (Koontz et al., 2012a). Body weight (Table 2) was not different between treatments as a result of pairing. Feed intake differed by design between H (1.8xNEₘ) and L (1.1xNEₘ) and did not differ between endophyte treatments due to pair feeding. Thus main effect differences between E+ and E- animals are due to alkaloid ingestion, not variation in feed intake.

As expected, H fed animals had higher water consumption ($P = 0.01$) than did L fed animals, whereas water consumption was not affected by endophyte treatment ($P = 0.74$). Water intake is known to rise with increased DM intake (Winchester and Morris, 1956b). While most fescue research has not measured water intake, those that have typically only see changes at elevated environmental temperature, without separating the effects of temperature and feed intake from that of endophyte treatment (Jackson et al., 1984a; Peters et al., 1988; Aldrich et al., 1993b). In agreement with the current experiment, Hannah et al (1990) showed no difference in water intake between endophyte treatments with equivalent OM intakes at thermoneutral temperature.

In the present study, heart rate was reduced ($P = 0.047$) by ingestion of endophyte infected seed and elevated ($P = 0.01$) by high feed intake. Numerous studies have shown a reduction in heart rate with consumption of endophyte-infected tall fescue (Osborn et al., 1992; Browning and Leite-Browning, 1997; Aiken et al., 2007). While these studies did not use pair fed animals, there was not a significant difference in feed intake between endophyte treatments. It has been postulated that the heart rate of animals is not directly affected by the alkaloids, but rather is reduced in response to vasoconstriction, allowing for the maintenance of a constant blood pressure (Oliver, 2005). The increased heart rate observed in H fed animals was anticipated as heart rate increases in relation to dietary intake (Brosh et al., 1998a).
Core temperature increased ($P < 0.0001$) with H feeding and E+ dosing. Increased dietary intake causes an increase in heat of fermentation and energy expenditures related to the digestive process which increases core temperature (Conrad, 1985). As with decreased heart rate, increased core temperature is a classic indicator of fescue toxicosis in cattle (Boling et al., 1989; Strickland et al., 2011). This increase is attributed to α-adrenergic receptor mediated vasoconstriction in subcutaneous areas (Dyer, 1993; Oliver, 1997) and the resulting reduced blood flow in subcutaneous tissues (Rhodes et al., 1991) reducing evaporative heat loss, thereby contributing to increased core body temperature.

5.3.2) DM Digestibility and In Situ Disappearance

Total tract DM digestibility was increased (Table 3, $P = 0.017$) with H feeding in the present experiment. While increased DM intake typically depresses digestibility due to increased passage rates (Church, 1988), this depression is minimal for diets composed solely of forage (Tyrrell and Moe, 1975). Several previous studies have also reported greater DM digestion with higher intakes in animals consuming un-infected fescue (Aldrich et al., 1993b; Humphry et al., 2002; Matthews et al., 2005).

The data from the present experiment indicate that the presence of alkaloids in the rumen from endophyte infected tall fescue do not affect ($P = 0.27$) total tract DM digestibility of alfalfa (Table 3). In agreement with this, Corrigan (2005) reported that source of rumen fluid (cattle grazing E+ or E- pasture) had no effect on in vitro DM disappearance of alfalfa or other feedstuffs. Previous research examining the digestibility of tall fescue by comparing infected and uninfected forages or seeds has provided mixed results. Increased (Schmidt et al., 1982; Goetsch et al., 1987), decreased (Westendorf et al., 1993; Aldrich et al., 1993b; Matthews et al., 2005), and unchanged (Harmon et al., 1991; Forcherio et al., 1995) digestibilities have been reported. While co-seeding of legumes and/or supplemental feeds are common amelioration techniques for animals on endophyte infected fescue pastures (Smith et al., 1975), there is a
lack of research examining the digestibility of supplemental feedstuffs in animals consuming endophyte infected fescue; or of ruminal degradation of a common feedstuff in animals consuming endophyte infected and uninfected diets. The results from the present study suggest that alkaloid ingestion does not alter the ability of the animal to digest other feedstuffs.

No differences ($P > 0.3$) were found between intake levels or endophyte treatments for *in situ* extent or rate of disappearance of ground alfalfa in the present study. Previous research has reported no difference (Flores et al., 2007) in ruminal *in situ* DM disappearance of E+ compared to a novel endophyte fescue. In contrast, reduced *in situ* DM disappearance for alfalfa and corn has been reported in cattle grazing E+ vs. E- pastures (Corrigan, 2005) and increased DM disappearance has been reported for cattle fed E+ and E- hay (Humphry et al., 2002). In the present experiment extent of *in situ* DM disappearance was greater than total tract DM. This lack of relationship is similar to that seen by Humphry et al. (2002) where heifers consuming infected fescue hay had reduced total tract DM digestion despite increased *in situ* DM disappearance as compared to heifers consuming an E- diet.

5.3.3) Nitrogen Balance

By design, N intake differed between feeding levels, but not endophyte treatments (Table 4). Nitrogen digestibility was unaffected ($P > 0.15$) by either endophyte treatment or intake. Both fecal and urinary N excretions were higher ($P < 0.0001$) for H fed animals. There was no difference ($P = 0.90$) in fecal N excretion between endophyte treatments; however, there was a tendency ($P = 0.11$) for increased N lost in urine with E+ dosing. Digestible N, retained N and N retained as a percent of intake were greater ($P < 0.0001$) for H fed animals, but not different ($P > 0.2$) between endophyte treatments. This differs from previous reports of animals consuming fescue hay, where E+ fed animals had lower N retention (Fiorito et al., 1991; Matthews et al., 2005). Matthews et al. (2005) did not hold intake equal between treatments, however, Fiorito et al. (1991) did. In both cases, when intake was accounted for by expressing N retention as a
percent of N intake, E+ animals were reported to have lower retention; whereas
the present study had no difference in retained N as a percent of intake between
endophyte treatments. This may be related to experimental design, as the
current experiment utilized a common feedstuff and ruminally dosed fescue seed,
providing a greater separation of feed and alkaloid intake and minimizing
differences in diet digestibility due to feed form and composition. The previous
research utilized E+ and E- hay as the diet and source of alkaloids. This may
indicate that previously reported differences in N retention during fescue toxicosis
are related differences in feedstuff composition and reduced digestion rather than
efficiency of utilization or endophyte presence.

5.3.4) Energy Balance

Energy intake, as well as fecal and urinary energy excretions were greater
($P < 0.001$; Table 5) for H fed animals, but not affected ($P > 0.15$) by endophyte
treatment. On average, L fed animals were slightly below maintenance, based
on an average RE of -111kJ/kg BW$^{75}$, despite consuming 1.09% of calculated
maintenance requirements. This indicates that for these animals, calculated
maintenance requirements were lower than actual requirements. Energy
digestibility was unchanged ($P = 0.37$) by endophyte treatment and tended to
increase ($P = 0.06$) with H feeding. There were no differences ($P > 0.36$) in DE,
ME, or RE between endophyte treatments, but these were increased ($P < 0.001$)
by H feeding, as expected. It is accepted that fecal and urinary energy excretion
increase with increased intake (Geay, 1984; Van Soest, 1994). Reid et al. (1980)
stated that in all forage diets digestibility is unaffected or only slightly affected
with increased intake, as digestibility is more closely associated with particle size.
In agreement with the present experiment, Iason and Murray (1996) found that
sheep dosed with plant phenolic compounds had no change in whole body
energy use or fecal energy output. These data suggest that energy retention
differences during fescue toxicosis are most likely a result of reduced intake.

Animals consumed feed at 1.09 and 1.74 ± 0.02 times the calculated
maintenance requirements respectively for the L and H intake levels on average.
Three animals had negative RE values at both dietary intake levels, and one animal had a lower RE at H intake as compared to L. These animals were considered outliers (one E-, three E+) and removed from the data set for evaluation of partial efficiency of ME use (Figure 1). There were no differences ($P = 0.24$) in partial efficiency between endophyte treatments, indicating that E+ dosing does not affect the ability of the animal to utilize available energy. Utilizing calculated N and E retention to determine composition of tissue gain or loss (McLeod et al., 2000) presented no difference ($P = 0.18$) between endophyte treatments for protein, while there was a tendency ($P = 0.10$) for greater adipose loss with E+ treatment (data not shown). In agreement with this, ADG was reduced by E+ seed inclusion in the diet of pair fed rats (Mizinga et al., 1993) and cows (Mizinga et al., 1990; 1992), though milk production was not affected by endophyte treatment of cows (Mizinga et al., 1992). In contrast, no difference in ADG due to fescue seed endophyte status was found in pair fed rats (Neal and Schmidt, 1985a) and rabbits (Filipov et al., 1998). Further, Stamm et al. (1994) reported no difference in feed efficiency of steers consuming either E+ or E- fescue straw.

Oxygen consumption and CO$_2$ production were increased ($P < 0.0001$) with H feeding regardless of endophyte treatment. However, RQ was not altered by level of intake ($P < 0.845$), indicating that the increases in oxygen consumption and carbon dioxide production were equivalent. Methane production did not differ between intake ($P = 0.168$) or endophyte ($P = 0.255$) treatments. This is in agreement with previous research where no difference in methane production was reported between endophyte treatments (Pavao-Zuckerman et al.; Aldrich et al., 1993b; Al-Haidary et al., 2001). This resulted in increased ($P < 0.0001$) heat production for H fed animals, but no effect of endophyte treatment ($P = 0.828$). In agreement with the present research, Aldrich et al. (1993b) reported no difference in heat production between endophyte treatments in steers fed ad libitum receiving slightly higher levels of ergovaline (0.021 mg / kg BW$^{75}$ vs. 0.012 mg / kg BW$^{75}$). Similarly, heifers fed an E+ diet (5 µg ergovaline / kg • d$^{-1}$) had no change in heat production at either
21°C or 31°C (Al-Haidary et al., 2001). Conversely, a reduction in heat production was reported for lambs consuming a diet containing a high level of ergovaline (0.118 mg / kg BW\textsuperscript{75}) at 32°C (Aldrich et al., 1993a). This may indicate that changes in heat production and energy use due to fescue toxicosis only occur with high levels of alkaloid intake and are most pronounced at elevated environmental temperatures.

The interaction of intake and endophyte was significant \((P = 0.04)\) for CO\textsubscript{2} production, and tended to be significant \((P \leq 0.12)\) for O\textsubscript{2} consumption, CH\textsubscript{4} production, and HP. For each of these, the difference between intake levels was greater during E+ dosing than E- dosing. Previous work reported a reduction in fasting heat production due to E+ ingestion, indicating a reduction in basal metabolic rate (Koontz et al., 2012b). In the present study, L feeding reduced HP, which was further depressed by E+ dosing. This may indicate that alkaloid ingestion may cause animals to be more efficient when intake is near maintenance due to reduced metabolic activity. However, when intake is increased above maintenance, the increased energy costs associated with elevated respiration rate, thermoregulation, and up-regulation of detoxification pathways during fescue toxicosis (Zanzalari et al., 1989; Settivari et al., 2006) may be prioritized over energy partitioned for weight gain, especially at elevated environmental temperatures, resulting in the reduced growth observed during fescue toxicosis and increased heat production reported here.

5.3.5) Ruminal Passage Rates

As expected, H feeding increased total rumen contents weight \((P = 0.0007; \text{Table 6})\) and tended to increase percent DM \((P = 0.101)\), resulting in a larger ruminal liquid volume \((P = 0.076)\) in L fed animals. As seen previously (Koontz et al., 2012b), dosing with E+ significantly increased the DM percentage \((P = 0.0007)\) and total DM weight \((P = 0.014)\) of rumen contents, while total weight of rumen contents \((P = 0.528)\) and liquid volume \((P = 0.442)\) were not different between endophyte treatments. This is in agreement with previous work by Aldrich et al. (1993a) where E+ fed sheep had no difference in ruminal fluid
volume despite reduced DM intake. In contrast, Hannah et al. (1990) showed increasing ergovaline in the diet of lambs resulted in decreased ruminal fluid volume with no difference in OM intake. In contrast, Stamm et al. (1994) reported a reduction in DM fill with no difference in liquid fill in the rumen of steers consuming E+ fescue straw, as compared to E-.

Both particulate flow rate and passage rate were increased ($P < 0.001$) by H feeding level in the present study, as expected. Particulate passage was reduced ($P = 0.02$) by E+ dosing in the present experiment. Stamm et al. (1994) also reported a decrease in particulate outflow with increasing levels of E+ straw in the diet of steers with similar DM intakes. $\alpha$-Adrenergic and serotonergic receptors have been implicated in control of GIT motility (Talley, 1992; van Miert et al., 1994); both of these receptor types are acted on by alkaloids present in endophyte-infected tall fescue (Larson et al., 1999; Schoning et al., 2001). McLeay and Smith (McLeay and Smith, 2006) found that IV administration of ergot alkaloids reduced ruminal contraction rates, which would alter particulate passage. The present results differ from previous research reporting no difference in particulate passage due to endophyte presence in the diet both with (Hannah et al., 1990) and without (Forcherio et al., 1995) equal intakes. However, the results from the present experiment explain the increase in ruminal DM content in this and previous research utilizing pair-fed animals (Foote et al., 2012b; Koontz et al., 2012b). This reduced particulate passage may also be an underlying cause of intake depression during fescue toxicosis. As increased ruminal fill causes distension which activates the satiety centers of the brain, reducing intake (Church, 1988).

Liquid passage rate from the rumen was increased ($P = 0.025$) with H feeding in the present study. This is most likely a result of increased water intake by H fed animals, which is known to drive liquid passage rate. While liquid outflow tended to be lower with E+ dosing ($P = 0.136$), the higher liquid passage rate of H fed animals tended to be further increased by E+ dosing of H fed animals ($P = 0.09$), which likely led to the increased ruminal DM. Hannah et al. (1990) reported reductions in both liquid flow and passage rates for animals with
equal OM intakes consuming E+ seed. However other research with reduced intakes due to E+ have reported reduced liquid passage without altered outflow rate or water intake (Aldrich et al., 1993a) while others have reported no difference in liquid passage rate (Goetsch et al., 1987; Stamm et al., 1994; Forcherio et al., 1995).

5.3.6) Ruminal VFA Concentrations

Increased intake by H animals resulted in greater average total VFA concentration \( (P = 0.005, \text{Figure 2}) \) than L fed animals. Ruminal VFA concentration was also affected by time of sample, with the highest levels seen after feeding (Figure 2). To account for the large difference in total rumen liquid volume, total VFA quantity present in the rumen was evaluated (data not shown). Animals fed at 1.1xNE\text{m} had a higher \( (P = 0.036) \) quantity of VFA regardless of endophyte treatment, likely due to a reduction in VFA absorption due to increased liquid volume (Dijkstra et al., 1993).

Animals dosed with E+ had greater average total VFA concentrations \( (P < 0.0001) \) and lower average ruminal pH \( (P = 0.001; \text{Figure 3}) \) as compared to E-dosed animals. Total VFA quantity corrected for ruminal liquid volume was not different \( (P = 0.41, \text{data not shown}) \) between endophyte treatments. As ruminal pH remained above 6.0 for all animals through the measurement period, this difference was not considered to be physiologically relevant and is likely only a reflection of the increased VFA concentration. Similar to the present research, E+ diet in animals with similar intakes reported increased total ruminal VFA concentration, with the proportions of acetate reduced and butyrate increased (Stamm et al., 1994). Alkaloid presence in the rumen has been shown to decrease blood flow to the rumen epithelium and acetate flux (Foote et al., 2012b). Thus the increase in total VFA concentration in the rumen of E+ dosed steers may be due to reduced absorption and not increased production by rumen microbes.

With the exception of valerate, molar proportions of individual VFA were altered by both intake level and endophyte treatment (Figure 4). On average,
acetate and propionate were increased \((P < 0.05)\) by H feeding, while average proportions of butyrate and the branched chain VFA (isobutyrate and isovalerate) were decreased \((P < 0.0001)\) with H feeding. Ruminal dosing of E+ seed resulted in increased proportion of butyrate \((P < 0.0001)\) and decreased the proportions of other VFA \((P < 0.05)\) with the exception of valerate which was unchanged \((P = 0.677)\). In agreement with the present study, Hannah et al (1990) reported increased total ruminal VFA with increasing dietary ergovaline in sheep, though no difference in molar proportions of individual VFA were found. Conversely, Harmon et al (1991) reported no differences in total VFA concentrations, with a decrease in the proportion of butyrate. In vitro fermentation with alkaloids from ryegrass also showed no change in total VFA, but proportions of acetate, propionate, and butyrate were increased (Silley, 1986). Further, experiments with perloline alkaloids from tall fescue increased total VFA concentration, with equivocal results concerning individual VFA. Proportion of acetate decreased in vivo (Boling et al., 1975) and was both unchanged (Bush et al., 1972) and reduced (Bush et al., 1976) in vitro with perloline addition, while propionate proportion was increased in vivo (Boling et al., 1975) and was both decreased (Bush et al., 1972) and increased (Bush et al., 1976) in vitro. In the present study, the increase in butyrate was greatest for the E-L treatment, which also showed the lowest CH4 production, indicating an alteration in the fate of hydrogen and availability to the animal. These data suggest that rumen fermentation and VFA production during fescue toxicosis needs further study to understand if the changes in profile are consistent and attributable to alkaloid ingestion.

5.4) Conclusion

Ingestion of endophyte infected tall fescue seed in pair fed steers does not alter total N or energy balance at high or low intake levels. Endophyte treatment also does not affect in situ degradation of a common feedstuff. Rumen contents %DM and DM weight is increased with E+ ingestion, which is likely related to reduced particulate passage from the rumen. This reduction in
particulate passage may drive the depression in intake common during fescue toxicosis by increasing distension. The ruminal VFA profile is altered by alkaloid ingestion, which may be due to reduced absorption, not increased production. These data indicate that the reduction in weight gain and productivity seen during fescue toxicosis is primarily a function of reduced intake and not secondary effects of alkaloid ingestion.
<table>
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<th></th>
<th>Alfalfa Cubes</th>
<th>Trace Mineral Salt Premix</th>
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Table 5.2: Animal weight, feed intake, heart rate and core temperature in steers dosed with endophyte free (E-)
and endophyte infected (E+) tall fescue seed fed at 1.1xNE\textsubscript{m} and 1.8xNE\textsubscript{m}\textsuperscript{1}

<table>
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<tr>
<td>Feed Intake (g DM/kg BW\textsuperscript{0.75})</td>
<td>3.94</td>
<td>6.32</td>
<td>3.94</td>
<td>6.32</td>
</tr>
<tr>
<td>Water Intake (L/kg BW\textsuperscript{0.75})</td>
<td>6.83</td>
<td>8.72</td>
<td>6.36</td>
<td>8.72</td>
</tr>
<tr>
<td>Heart Rate (beats/min)</td>
<td>73.2</td>
<td>88.3</td>
<td>70.6</td>
<td>76.7</td>
</tr>
<tr>
<td>Core Temperature (°C)</td>
<td>38.05</td>
<td>38.32</td>
<td>38.48</td>
<td>38.92</td>
</tr>
</tbody>
</table>

\textsuperscript{1} Data are presented as least squares means of animals dosed with E+ and E- treatments at each dietary intake level (n = 6).

\textsuperscript{2} Probability of a greater F statistic
Table 5.3: Diet DM digestibility and *in situ* rate, extent of degradation, and fractions (A, B, and C) of ground alfalfa in the rumen of steers dosed with endophyte free (E-) and endophyte infected (E+) tall fescue seed fed at 1.1xNE\(_m\) and 1.8xNE\(_m\),\(^{1,2}\)

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment</th>
<th>(1.1\times\text{NE}_m)</th>
<th>(1.8\times\text{NE}_m)</th>
<th>(1.1\times\text{NE}_m)</th>
<th>(1.8\times\text{NE}_m)</th>
<th>SEM</th>
<th>(P = )(^{3}) Main Effects</th>
<th>(P = )(^{3}) Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Tract DM digestibility</td>
<td>(%)</td>
<td>51.3</td>
<td>55.9</td>
<td>50.2</td>
<td>53.7</td>
<td>1.81</td>
<td>0.270</td>
<td>0.017</td>
</tr>
<tr>
<td>(%) In Situ Rate</td>
<td></td>
<td>11.2</td>
<td>9.6</td>
<td>15.8</td>
<td>12.9</td>
<td>6.35</td>
<td>0.366</td>
<td>0.632</td>
</tr>
<tr>
<td>(%) In Situ Extent</td>
<td></td>
<td>71.68</td>
<td>76.20</td>
<td>75.27</td>
<td>73.99</td>
<td>2.733</td>
<td>0.701</td>
<td>0.349</td>
</tr>
<tr>
<td>A fraction (%, \text{rapidly degraded})</td>
<td></td>
<td>32.68</td>
<td>32.97</td>
<td>33.78</td>
<td>33.01</td>
<td>1.349</td>
<td>0.517</td>
<td>0.805</td>
</tr>
<tr>
<td>B fraction (%, \text{slowly degraded})</td>
<td></td>
<td>46.75</td>
<td>44.89</td>
<td>41.39</td>
<td>47.66</td>
<td>6.764</td>
<td>0.744</td>
<td>0.668</td>
</tr>
<tr>
<td>C fraction (%, \text{undegraded})</td>
<td></td>
<td>24.26</td>
<td>23.04</td>
<td>21.05</td>
<td>19.18</td>
<td>5.450</td>
<td>0.322</td>
<td>0.675</td>
</tr>
</tbody>
</table>

\(^{1}\) Data presented as least squares means of animals dosed with E+ and E- treatments at each dietary intake level \(n = 6\).

\(^{2}\) According to the model \(p = a + b \times (1 - e^{-ct})\)

\(^{3}\) Probability of a greater F statistic
Table 5.4: Nitrogen balance (g / kg BW$^{75}$ d$^{-1}$) of steers dosed with endophyte free (E-) and endophyte infected (E+) tall fescue seed fed at 1.1xNE$_m$ and 1.8xNE$_m$$^1$

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment</th>
<th>$P = 2$</th>
<th>SEM</th>
<th>Main Effects</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E-</td>
<td>E+</td>
<td></td>
<td>Endophyte (E)</td>
<td>Intake (I)</td>
</tr>
<tr>
<td>Intake N</td>
<td>1.85</td>
<td>2.96</td>
<td>1.84</td>
<td>2.95</td>
<td>0.082</td>
</tr>
<tr>
<td>N digestibility (%)</td>
<td>65.7</td>
<td>68.2</td>
<td>66.1</td>
<td>67.4</td>
<td>1.23</td>
</tr>
<tr>
<td>Fecal N</td>
<td>0.64</td>
<td>0.93</td>
<td>0.63</td>
<td>0.95</td>
<td>0.032</td>
</tr>
<tr>
<td>Digestible N</td>
<td>1.21</td>
<td>2.02</td>
<td>1.21</td>
<td>1.99</td>
<td>0.071</td>
</tr>
<tr>
<td>Urinary N</td>
<td>1.24</td>
<td>1.45</td>
<td>1.30</td>
<td>1.50</td>
<td>0.079</td>
</tr>
<tr>
<td>Retained N</td>
<td>-0.03</td>
<td>0.57</td>
<td>-0.09</td>
<td>0.49</td>
<td>0.101</td>
</tr>
<tr>
<td>RN (% of IN)</td>
<td>-1.25</td>
<td>19.7</td>
<td>-4.94</td>
<td>16.8</td>
<td>4.24</td>
</tr>
</tbody>
</table>

$^1$ Data are presented as least squares means of animals dosed with E+ and E- treatments at each dietary intake level (n = 6).

$^2$ Probability of a greater F statistic
Table 5.5: Energy balance (kJ / kg BW$^{.75}$ d$^{-1}$) of steers dosed with endophyte free (E-) and endophyte infected (E+) tall fescue seed fed at 1.1xNE$_m$ and 1.8xNE$_m$.

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment</th>
<th>P $^{2}$</th>
<th>SEM</th>
<th>Main Effects</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E-</td>
<td>E+</td>
<td></td>
<td>Endophyte (E)</td>
<td>Intake (I)</td>
</tr>
<tr>
<td>Intake E</td>
<td>1118.3</td>
<td>1793.0</td>
<td>1112.0</td>
<td>1785.4</td>
<td>49.70</td>
</tr>
<tr>
<td>E digestibility (%)</td>
<td>51.0</td>
<td>55.2</td>
<td>49.6</td>
<td>53.3</td>
<td>2.58</td>
</tr>
<tr>
<td>Fecal E</td>
<td>546.8</td>
<td>805.5</td>
<td>559.0</td>
<td>834.2</td>
<td>27.70</td>
</tr>
<tr>
<td>Urinary E</td>
<td>60.5</td>
<td>76.7</td>
<td>63.4</td>
<td>84.8</td>
<td>5.47</td>
</tr>
<tr>
<td>Methane E</td>
<td>67.6</td>
<td>66.4</td>
<td>54.5</td>
<td>68.8</td>
<td>7.04</td>
</tr>
<tr>
<td>Digestible E</td>
<td>572.5</td>
<td>986.6</td>
<td>553.9</td>
<td>950.3</td>
<td>53.63</td>
</tr>
<tr>
<td>Metabolizable E</td>
<td>446.0</td>
<td>841.9</td>
<td>437.6</td>
<td>795.1</td>
<td>50.85</td>
</tr>
<tr>
<td>O$_2$ consumed</td>
<td>27.69</td>
<td>32.11</td>
<td>26.12</td>
<td>33.23</td>
<td>0.946</td>
</tr>
<tr>
<td>CO$_2$ produced</td>
<td>26.02</td>
<td>30.13</td>
<td>24.65</td>
<td>31.57</td>
<td>0.840</td>
</tr>
<tr>
<td>RQ (L CO$_2$/L O$_2$)</td>
<td>0.950</td>
<td>0.936</td>
<td>0.947</td>
<td>0.948</td>
<td>0.022</td>
</tr>
<tr>
<td>CH$_4$ produced</td>
<td>1.708</td>
<td>1.678</td>
<td>1.377</td>
<td>1.739</td>
<td>0.178</td>
</tr>
<tr>
<td>Heat Production E</td>
<td>574.4</td>
<td>667.2</td>
<td>542.9</td>
<td>692.3</td>
<td>18.47</td>
</tr>
<tr>
<td>Retained E</td>
<td>-123.5</td>
<td>169.8</td>
<td>-100.3</td>
<td>97.9</td>
<td>55.55</td>
</tr>
</tbody>
</table>

$^1$ Data are presented as least squares means of animals dosed with E+ and E- treatments at each dietary intake level (n = 6).

$^2$ Probability of a greater F statistic.
Table 5.6: Rumen contents weight, dry mater, fluid volume, and liquid passage rate of steers dosed with endophyte free (E-) and endophyte infected (E+) tall fescue seed fed at 1.1xNE\textsubscript{m} and 1.8xNE\textsubscript{m}\textsuperscript{1,2}

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment</th>
<th>SEM</th>
<th>$P = ^{2}$</th>
<th>Main Effects</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Item</td>
<td>E-</td>
<td>E+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rumen Contents (g/kg BW\textsuperscript{75})</td>
<td>636.9</td>
<td>726.1</td>
<td>616.7</td>
<td>720.0</td>
<td>28.72</td>
</tr>
<tr>
<td>Rumen Contents (%DM)</td>
<td>10.8</td>
<td>12.2</td>
<td>13.3</td>
<td>13.5</td>
<td>0.59</td>
</tr>
<tr>
<td>Rumen Contents (g DM/kg BW\textsuperscript{75})</td>
<td>850.3</td>
<td>761.4</td>
<td>1006.6</td>
<td>834.1</td>
<td>44.85</td>
</tr>
<tr>
<td>Particulate Flow Rate (kg DM/h)</td>
<td>2.82</td>
<td>4.46</td>
<td>2.91</td>
<td>4.54</td>
<td>0.167</td>
</tr>
<tr>
<td>Particulate Passage Rate (%/h)</td>
<td>1.98</td>
<td>2.35</td>
<td>1.67</td>
<td>2.17</td>
<td>0.096</td>
</tr>
<tr>
<td>Liquid Volume (L)</td>
<td>51.1</td>
<td>32.2</td>
<td>44.7</td>
<td>23.3</td>
<td>10.95</td>
</tr>
<tr>
<td>Liquid Flow Rate (L/h)</td>
<td>4.53</td>
<td>3.02</td>
<td>2.48</td>
<td>2.53</td>
<td>0.851</td>
</tr>
<tr>
<td>Liquid Passage Rate (%/h)</td>
<td>8.75</td>
<td>9.45</td>
<td>7.74</td>
<td>11.37</td>
<td>1.059</td>
</tr>
</tbody>
</table>

\textsuperscript{1} Data presented as least squares means of animals dosed with E+ and E- treatments at each dietary intake level (n = 6).

\textsuperscript{2} Probability of a greater F statistic
Figure 5.1. Partial efficiency of steers dosed with endophyte free (E-, n=5) and endophyte infected (E+, n=3). Calculated as the slope of the line between the ratio of RE to ME at each intake (1.1xNE_m and 1.8xNE_m) during each endophyte treatment ($P = 0.240$)
Figure 5.2. Total ruminal VFA concentration of steers dosed with endophyte free (E-) and endophyte infected (E+) tall fescue seed fed at 1.1xNEm (L) and 1.8xNEm (H) over a 24h sampling period. Steers were fed and dosed with ground fescue seed immediately following the 0h sampling point and at 8h. Fixed effect p-values for endophyte treatment (E), intake level (I), time (T) and their interactions are shown for each figure. Significant differences between endophyte treatment (E- vs. E+) within T are denoted by * ($P < 0.05$). Significant differences between intake levels (L vs. H) within T are denoted by $#$ ($P < 0.05$).
Figure 5.3. Ruminal pH of steers dosed with endophyte free (E-) and endophyte infected (E+) tall fescue seed fed at 1.1xNEm (L) and 1.8xNEm (H) over a 24h sampling period. Steers were fed and dosed with ground fescue seed immediately following the 0h sampling point and at 8h. Fixed effect p-values for endophyte treatment (E), intake level (I), time (T) and their interactions are shown for each figure. Significant differences between E (E- vs. E+) within T are denoted by * ($P < 0.05$). Significant differences between I (L vs. H) within T are denoted by $\#$ ($P < 0.05$).
Figure 5.4. Molar proportions of acetate (A), propionate (B), isobutyrate (C), butyrate (D), isovalerate (E), and valerate (F) in rumen fluid of steers (n=6) dosed with endophyte free (E-) and endophyte infected (E+) tall fescue seed fed at 1.1xNEm (L) and 1.8xNEm (H) over a 24h sampling period. Where E-L = ■, E-H = □, E+L = ●, and E+H = ○. Steers were fed and dosed with ground fescue seed immediately following the 0h sampling point and at 8h. Fixed effect p-values for endophyte treatment (E), intake level (I), time (T) and their interactions are shown for each figure. Significant differences between E (E- vs. E+) within T are denoted by * \((P < 0.05)\). Significant differences between I (L vs. H) within T are denoted by # \((P < 0.05)\).
A. Acetate

- E: P < 0.0001
- I: P < 0.0001
- T: P = 0.008
- E x I: P = 0.005
- E x T: P = 0.005
- I x T: P = 0.005

B. Propionate

- E: P = 0.046
- I: P = 0.002
- T: P = 0.0002
- E x I: P < 0.0001
- E x T: P = 0.934
- I x T: P = 0.82

C. Isobutyrate

- E: P < 0.0001
- I: P = 0.007
- T: P < 0.0001
- E x I: P = 0.003
- E x T: P < 0.0001
- I x T: P = 0.897

D. Butyrate

- E: P < 0.0001
- I: P < 0.0001
- T: P < 0.0001
- E x I: P = 0.0003
- E x T: P = 0.438
- I x T: P = 0.120

E. Isobutyrate

- E: P < 0.0001
- I: P < 0.0001
- T: P < 0.0001
- E x I: P = 0.033
- E x T: P < 0.0001
- I x T: P = 0.944

F. Valerate

- E: P = 0.677
- I: P = 0.403
- T: P = 0.008
- E x I: P = 0.978
- E x T: P = 0.132
- I x T: P = 0.597
Endophyte infected tall fescue is widely planted in the Southeast due to its heat and drought tolerance, insect resistance, and ability to withstand intensive grazing. These desirable characteristics are largely provided by the symbiotic relation with the fungal endophyte *Neotyphodium coenophialum*. These benefits often come with the drawback of reducing intake and weight gain by animals consuming the alkaloids produced by the endophyte, predominantly during summer months.

Many of the secondary effects of fescue toxicosis such as cardiovascular changes, vasoconstriction, and reproductive problems have been well characterized. However, the most significant problems associated with fescue toxicosis are correlated with increased environmental temperature and reduced feed intake. Comparatively little research has been conducted to separate the effects resulting from each of these factors. The goal of the research presented in this dissertation was to separate these aspects in order to determine the effect of alkaloid ingestion on nutrient balance, energy metabolism, and digestion.

The primary experiment validated a novel method to induce fescue toxicosis that allowed for separation of alkaloid intake from feed intake. Results from this experiment also indicated that effects of alkaloid intake vary with differing environmental temperature. Utilization of this method allows for experiments designed to investigate the discrete effects and interactions of alkaloid ingestion, reduced feed intake, and elevated environmental temperature.

In the following experiments, ingestion of ergot alkaloids was found to reduce fasting heat production without alteration of whole body energy balance. Further, nitrogen balance was unaffected by alkaloid intake. The data from this research leads to the conclusion that ergot alkaloids from fescue do not alter the total nutrient utilization, but may affect nutrient and energy partitioning. Alkaloid ingestion may cause animals to be more efficient when intake is near maintenance due to reduced metabolic activity. However, when intake is increased above maintenance, preferential nutrient use by specific processes
such as respiration, detoxification, and thermoregulation may occur during fescue toxicosis, especially at elevated environmental temperatures, reducing energy and nutrient availability for animal growth.

The research from this dissertation also indicates that ergot alkaloids do not alter DM degradation of feed when separated from intake and environmental temperature. As a common amelioration technique for fescue toxicosis is supplementation and co-seeding with legumes, this indicates that digestion and utilization of these feedstuffs is not compromised by simultaneous fescue alkaloid consumption. Ingestion of alkaloids also increased ruminal VFA concentrations. Based on previous research, this is likely due to reduced absorption as opposed to increased production by ruminal microbes.

This research points to several future directions. We report a lack of alteration in whole-body nitrogen and energy balance due to alkaloid consumption at thermoneutral temperatures in pair fed animals. The majority of animals consuming endophyte infected tall fescue pasture do so during the summer and heat stress is known to alter nutrient metabolism. It would be of interest to determine if the alteration of nutrient use during heat stress is exacerbated by alkaloid ingestion, thus contributing to the increased severity of fescue toxicosis in summer months.

The increase in ruminal dry-matter of animals dosed with endophyte-infected fescue and corresponding reduction in particulate passage and tendency for increased liquid passage, provide evidence that ruminal fill is a primary driver of the reduction in intake seen during fescue toxicosis. Future research examining ruminal contraction rates and rate of intake relative to ruminal content composition could shed more light on this observation. Also, if the increase in ruminal VFA concentration reported here is due to decreased absorption, related to reduced blood flow, examination of methods to increase blood flow to the rumen epithelium may provide sufficient increased nutrient availability to compensate for altered partitioning.

In addition, the model developed and utilized by this research presents a new method for determining the metabolic, genomic, and physiological effects of
alkaloid ingestion. Previous research has often been confounded by intake, feedstuff, and/or temperature. This model minimizes such differences across treatments allowing for improved investigation of alterations which occur during fescue toxicosis. As ergot alkaloids are still used in many parts of the world for human medical applications, continued analysis of the metabolic, genomic, and physiological effects of alkaloids may allow doctors to better treat illness and address complications related to alkaloid utilization.

Despite the fact that the effects of endophyte-infected tall fescue has on livestock have been studied for several decades, there are still areas in the research and understanding of the underlying mechanisms of production losses that have not been fully elucidated. The research in this dissertation has contributed to addressing the confounding effects of reduced feed intake and environmental temperature on fescue toxicosis. If the problems associated with each of these factors can be delineated, specific amelioration techniques can be devised to address the larger issue of fescue toxicosis. This would allow for improved animal performance without increasing input costs to the producer related to supplemental feeds, reseeding pastures with novel endophyte or uninfected fescues.
APPENDIX A: Determining Recovery Rates of Head Boxes

In order to evaluate the accuracy of the indirect calorimetry system, the recovery of O₂ and CO₂ was determined by a controlled burn of a known quantity of propane in the head-box. A tank of 99.5% pure propane (Scott-Gross Company, Inc, Lexington, KY) was placed on a scale external to the head-box. Flexible, non-reactive tubing was used to connect the propane tank to a burner inside the head-box. Oxymax software (Columbus Instruments, v4.54) was utilized to record the measurements of O₂ and CO₂. The software set to take a reading from inside a specific chamber at 1 minute intervals, with a reading of the outside/room air after each 10th interval. After lighting the burner, the head-box was sealed and the initial weight of the propane tank recorded. At 30 minute intervals over a 2 hour period, the weight of the propane tank and the O₂ and CO₂ accumulated (as reported by the Oxymax system) was recorded. This procedure was completed at least three times for each of the four chambers, with each chamber being tested on two non-consecutive days over a two week period. A summary of these measurements is provided in Table A.1.

The theoretical O₂ use was determined using the formula:

\[ \Delta C_3H_8 \div 44g \times 5 \times 22.4L \]

Where \( \Delta C_3H_8 \) is the weight change of the propane tank in grams, 44g is the molecular weight of propane, 5 is the number of O₂ molecules necessary for complete combustion of 1 mole of propane, and 22.4L is the volume of one mole of oxygen at standard temperature and pressure. Theoretical CO₂ production from combusting propane was calculated as:

\[ \Delta C_3H_8 \div 44g \times 3 \times 22.4L \]

Where \( \Delta C_3H_8 \) is the weight change of the propane tank in grams, 44g is the molecular weight of propane, 3 is the number of CO₂ molecules produced during the complete combustion of 1 mole of propane, and 22.4L the volume of one mole of oxygen at standard temperature and pressure.
These theoretical values were then compared to the values recorded by the Oxymax software. Percent recovery was calculated as:

\[
\text{(RecGas/TheoGas)} \times 100\%
\]

Where \( \text{RecGas} \) is the amount oxygen or carbon dioxide recovered, as reported by the Oxymax system and \( \text{TheoGas} \) is the theoretical oxygen used or carbon dioxide produced in the combustion. The system was found to recover \( \text{O}_2 \) at \( 105.8 \pm 4.8\% \) and \( \text{CO}_2 \) at \( 103.3 \pm 4.7\% \) on average across all chambers. Individual averages for each chamber are reported below and were used to correct \( \text{O}_2 \) and \( \text{CO}_2 \) values prior to heat production and energy use determinations in the experiments reported here.

Table A.1: Average Recovery of Oxygen and Carbon Dioxide from 2-hr Propane Combustions for Each of Three Head-Boxes in the Indirect Calorimetry System

<table>
<thead>
<tr>
<th>Chamber</th>
<th>( \text{O}_2 ) (%)</th>
<th>( \text{CO}_2 ) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chamber 2</td>
<td>107.5</td>
<td>105.4</td>
</tr>
<tr>
<td>Chamber 3</td>
<td>108.9</td>
<td>105.8</td>
</tr>
<tr>
<td>Chamber 4</td>
<td>101.1</td>
<td>98.6</td>
</tr>
<tr>
<td>Overall</td>
<td>105.8</td>
<td>103.3</td>
</tr>
</tbody>
</table>
APPENDIX B: Permission to Utilize Published Material

January 10, 2012
Dr. Steven A. Zinn
Editor in Chief
Journal of Animal Science

Dr. Zinn:
I am completing a doctoral dissertation at the University of Kentucky entitled "Effects of Endophyte Infected Fescue Alkaloid Ingestion on Energy Metabolism, Nitrogen Balance, In Situ Feed Degradation, and Ruminal Passage Rates." I would like permission to reprint as a chapter in my dissertation the previously publish article:


I will include an acknowledgment to the article on the first page of the chapter.

Thank you for your assistance,

Anne Koontz

January 18, 2013
Anne,

You have permission to use the article. Remember that JAS owns the copyright. Please insert the reference and indicate it is being published with permission.

Good luck with the dissertation.

Steven Zinn
Editor-in-Chief of the Journal of Animal Science
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Department of Animal Science
108 George White
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WORKS CITED


Dew, R. K. 1989. Effects of endophyte-infected tall fescue on cellular and humoral aspects of immune function in the rat, mouse, and bovine. , Univ. of Kentucky, Lexington, KY.


Fletcher, L. 2010. Novel endophytes in new zealand grazing systems - the perfect solution, or a compromise Mycological Society of America/International Symposium on Fungl Endophytes in Grasses. University of Kentucky, Lexington, KY.


Goff, B. M. 2012. Steer and tall fescue pasture responses to grazing intensity and chemical seedhead suppression, University of Kentucky Libraries, Lexington, Ky.


Hoveland, C. S. 1993. Importance and economic significance of the acremonium endophytes to performance of animals and grass plant. Agriculture, Ecosystems & Environment 44: 3-12.


performance and association of acremonium coenophialum fungal endophyte on tall fescue pasture. Agron J 75: 821-824.


Kennedy, P. B. 1900. Cooperative experiments with grasses and forage plants. USDA. Bull. 22.


Lacefield, G. D. 2006. Tall fescue from 1931-2006. In: Heart of America Grazing Conference, Cave City, KY


Mays, C. E. 2005. Effects of endophyte-infected fescue and feb-200 on reproductive performance of beef bulls, University of Kentucky, Lexington, KY.


Sharf, B. A. 2008. Comparison of thermoregulative mechanisms in heat sensitive and tolerant breeds of bos taurus cattle, University of Missouri, Columbia.


Stewart, R. L., G. Scaglia, O. A. Abaye, W. S. Swecker, E. A. Wong, M. McCann, and J. P. Fontenot. 2010. Tall fescue copper and copper-zinc superoxide dismutase status in beef steers grazing three different fescue types. The Professional Animal Scientist 26: 489-497.


Anne Fleming Koontz was born in Atlanta, GA on September 28, 1982. Anne received her Bachelor’s of Science in Animal Science, with an emphasis in biotechnology from Oklahoma State University in 2005. There she was involved in poultry production research under the supervision of Dr. Robert Teeter. She assisted in experiments related to minimizing energy use in broilers via alterations in feed form and lighting cycles, as well as commercial product evaluations. In 2005, she was selected for a Department of Homeland Security fellowship. During the summer of 2006, Anne worked at Texas A&M University with Dr. John El-Attrache. There she was involved in research related to early detection of influenza-A in feral swine and avian populations. During the summer of 2008 Anne was selected to be the first recipient of Alltech’s Margin of Excellence Fellowship. She received her Master’s of Science in 2009 from the University of Kentucky under Dr. Kyle McLeod. Her thesis was entitled *Effects of Ractopamine on Whole Body and Splanchnic Energy Balance in Holstein Steers*.

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