University of Kentucky
UKnowledge

Theses and Dissertations--Animal and Food Sciences

Animal and Food Sciences

2014

EFFECT OF DIETARY EXPOSURE TO ERGOT ALKALOIDS ON CONTRACTILITY OF BOVINE MESENTERIC VASCULATURE AND RUMEN MOTILITY

Amanda M. Egert
University of Kentucky, amandaegert@gmail.com

Right click to open a feedback form in a new tab to let us know how this document benefits you.

Recommended Citation

Egert, Amanda M., "EFFECT OF DIETARY EXPOSURE TO ERGOT ALKALOIDS ON CONTRACTILITY OF BOVINE MESENTERIC VASCULATURE AND RUMEN MOTILITY" (2014). *Theses and Dissertations--Animal and Food Sciences*. 40.
https://uknowledge.uky.edu/animalsci_etds/40

This Master's Thesis is brought to you for free and open access by the Animal and Food Sciences at UKnowledge. It has been accepted for inclusion in Theses and Dissertations--Animal and Food Sciences by an authorized administrator of UKnowledge. For more information, please contact UKnowledge@lsv.uky.edu.

STUDENT AGREEMENT:

I represent that my thesis or dissertation and abstract are my original work. Proper attribution has been given to all outside sources. I understand that I am solely responsible for obtaining any needed copyright permissions. I have obtained needed written permission statement(s) from the owner(s) of each third-party copyrighted matter to be included in my work, allowing electronic distribution (if such use is not permitted by the fair use doctrine) which will be submitted to UKnowledge as Additional File.

I hereby grant to The University of Kentucky and its agents the irrevocable, non-exclusive, and royalty-free license to archive and make accessible my work in whole or in part in all forms of media, now or hereafter known. I agree that the document mentioned above may be made available immediately for worldwide access unless an embargo applies.

I retain all other ownership rights to the copyright of my work. I also retain the right to use in future works (such as articles or books) all or part of my work. I understand that I am free to register the copyright to my work.

REVIEW, APPROVAL AND ACCEPTANCE

The document mentioned above has been reviewed and accepted by the student's advisor, on behalf of the advisory committee, and by the Director of Graduate Studies (DGS), on behalf of the program; we verify that this is the final, approved version of the student's thesis including all changes required by the advisory committee. The undersigned agree to abide by the statements above.

Amanda M. Egert, Student

Dr. David L. Harmon, Major Professor

Dr. David L. Harmon, Director of Graduate Studies

EFFECT OF DIETARY EXPOSURE TO ERGOT ALKALOIDS ON CONTRACTILITY OF BOVINE MESENTERIC VASCULATURE AND RUMEN MOTILITY

THESIS

A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Science in the
College of Agriculture, Food, and Environment
at the University of Kentucky

By

Amanda M. Egert

Lexington, Kentucky

Director: Dr. David L. Harmon, Professor of Animal Science

Lexington, Kentucky

2014

Copyright © Amanda M. Egert 2014

ABSTRACT OF THESIS

EFFECT OF DIETARY EXPOSURE TO ERGOT ALKALOIDS ON CONTRACTILITY OF BOVINE MESENTERIC VASCULATURE AND RUMEN MOTILITY

Endophyte-infected (E+) tall fescue grass has been associated with fescue toxicosis, a costly syndrome characterized by poor cattle performance and health resulting in significant production losses. The fungal endophyte produces ergot alkaloids, which help the grass thrive in poor conditions but are toxic to mammals. A number of symptoms of fescue toxicosis can be related to vasoconstriction of bovine core, peripheral, and foregut vasculature. The first part of this series of experiments demonstrated ergot alkaloids were also vasoactive in midgut vasculature, with the exception of lysergic acid. Additionally, prior dietary exposure to ergot alkaloids decreased the contractile response of mesenteric vasculature to many of the ergot alkaloids tested. In the second part of this series, a non-invasive method was developed for measuring rumen motility in cannulated cattle. Using this technology without different dietary treatments, it was determined that 8 to 16 h after feeding was the least variable between animals and would provide the best opportunity to measure differences in motility. Application of this technique in the third part of this series investigated the effect of ruminally dosed ergot alkaloids on rumen motility. Treatments were not effective at inducing fescue toxicosis, and no differences in rumen motility variables were detected.

KEYWORDS: tall fescue, fescue toxicosis, vasoconstriction, contractions, rumen pressure

Amanda M. Egert
Student's Signature

December 8th, 2014
Date

EFFECT OF DIETARY EXPOSURE TO ERGOT ALKALOIDS ON CONTRACTILITY OF BOVINE MESENTERIC VASCULATURE AND RUMEN MOTILITY

By

Amanda M. Egert

Dr. David L. Harmon
Director of Thesis

Dr. David L. Harmon
Director of Graduate Studies

December 8th, 2014
Date

ACKNOWLEDGMENTS

Firstly, I would like to thank my advisor, Dr. David Harmon, for providing me with this opportunity, supporting and directing my academic and research programs, and always pushing me to write and publish. I am extremely grateful for all of the helpful advice, encouragement, and knowledge that he provided while working towards my degree. A great deal of appreciation is also expressed towards committee members Dr. James Klotz, who has played an integral part of my research, and Dr. Kyle McLeod. The completion of my graduate program and thesis would not have been possible without their guidance, and I would like to thank them for their time.

I am grateful to Do Hyung Kim, Adam Barnes, and Winston Lin, as well as the entire University of Kentucky, C. Oran Little Research Center, Beef Unit farm staff for their aid in conducting these experiments. Specifically, Lauren Clark provided an immense amount of assistance caring for the animals, troubleshooting, and collecting data. Additionally, I would like to thank Ryan Chaplin and Dr. Gregg Rentfrow of the University of Kentucky Meats Lab. Appreciation is also expressed to the graduate students who were not afraid to get their hands dirty and help with rumen evacuations.

Thank you to the animals (particularly those that I cared for extensively: Baby, Whitey, Frankie, Buddy, Tiny, Pops, Burt, and Ernie) that were used in these experiments for cooperating, even though sometimes not initially.

Furthermore, I wish to extend an enormous thank you to my family and, most importantly, my parents, Kay and Dave. You were always there for me, listened, and offered words of inspiration when I needed them most. Words cannot describe the extent of my gratitude. Without your love and support, I would not be the woman I am today.

Finally, I would like to thank my friends, my cat, Hank, and my horse, Trip, for keeping me sane, reminding me that it is OK to have a life outside of graduate school, and understanding when I was too busy with experiments to spend time with them.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

FREQUENTLY USED ABBREVIATIONS

5HT	5-Hydroxytryptamine; serotonin
ADF	Acid Detergent Fiber
CP	Crude Protein
d	Day
DM	Dry Matter
DMI	Dry Matter Intake
E-	Endophyte-free
E+	Endophyte-infected
ERN	Ergonovine
ERO	Ergocornine
ERP	Ergocryptine
ERS	Ergocristine
ERT	Ergotamine
ERV	Ergovaline
EXT	Endophyte-infected tall fescue seed extract
GIT	Gastrointestinal tract
i.d.	Inner Diameter
KCl	Potassium Chloride
KY-31	Kentucky 31
KY-32	Kentucky 32
LSA	Lysergic Acid
MA	Mesenteric Artery
MV	Mesenteric Vein
min	Minute

NE_m	Net Energy of Maintenance
NDF	Neutral Detergent Fiber
o.d.	Outer Diameter
RT	Relaxation Time
TTP	Time to Peak
VFA	Volatile Fatty Acid

Chapter 1: Introduction

Neotyphodium coenophialum is a symbiotic fungal endophyte of tall fescue, a prominent cool season forage grass for animal production, particularly across the Southeastern and Pacific Northwest regions of the United States (Porter et al., 1979; Bush et al., 1982; Bacon and Siegel, 1988). Association of the grass with this endophyte confers desirable properties for the growth, persistence, and maintenance of the plant, making it a valuable forage. However, it is now known that the endophyte produces compounds which cause fescue toxicosis in animals that consume endophyte-infected tall fescue grass, hay, or seed (Lyons et al., 1986; Strickland et al., 2011). Fescue toxicosis is characterized by three syndromes, summer slump, fescue foot, and fat necrosis, which all result in decreased animal performance as evidenced by reduced gains, intake, reproduction efficiency, serum prolactin, etc. Due to the vast amount of acreage covered by tall fescue in the United States and over 50% of that being infected with *N. coenophialum*, the production losses related with endphyte-infected tall fescue consumption have been estimated over $1 billion annually for all livestock species (Strickland et al., 2011).

The primary compounds believed to be responsible for this syndrome are ergot alkaloids. Ergot alkaloids induce vasoconstriction in a variety of bovine vasculature types (Rhodes et al., 1991; Solomons et al., 1989; Klotz et al., 2006; Aiken et al., 2007; Foote et al., 2011), which could be related to the reduction in heat tolerance and gangrene of fescue toxicosis in hot and cold ambient temperatures, respectively. Additionally, blood flows to rumen epithelium (Foote et al., 2013), duodenum (Rhodes et al., 1991), and colon (Rhodes et al., 1991) were found to decrease, which suggests nutrient absorption may be altered as well. Until now, no research has been conducted

to investigate the effect of ergot alkaloids on midgut vasculature or the vasoactivity of ergot alkaloids on vasculature that has previously been exposed to ergot alkaloids. However, reduced blood flow to absorptive tissues could greatly affect the transfer of nutrients and therefore, nutrient availability to the animal.

Furthermore, ergot alkaloids consumed with endophyte-infected tall fescue consumption may alter gut motility. Westendorf et al. (1993) provided evidence of ergot alkaloids in the abomasum, but only 50 to 60% of that which was administered in the diet. Thus, motility of the rumen, which is proposed to be the primary site for ergot alkaloid metabolism and absorption, could be affected. For example, Foote et al. (2013) demonstrated that the DM percentage and dry contents of the rumen on a BW basis were greater for cattle that were ruminally dosed with endophyte-infected (**E+**) tall fescue seed compared to cattle dosed with endophyte-free (**E-**) seed. This finding could indicate a difference in particulate or liquid passage rates (not measured) which may be related to a decrease in motility. Particulate passage rate decreased and liquid flow rate showed a tendency to decrease with E+ seed dosing in another study (Koontz et al., 2014). Intravenous injection of ergot alkaloids, specifically ergotamine and ergovaline, in sheep has been shown to decrease forestomach motility (McLeay and Smith, 2006; Poole et al., 2009). Yet, research investigating the effect of ruminally administered ergot alkaloids or endophyte-infected tall fescue seed on rumen motility patterns in cattle is lacking.

Therefore, the aim of this thesis was to determine if 1) mesenteric vasculature would constrict with exposure to ergot alkaloids, 2) the contractile response to ergot alkaloids in vitro differs with previous exposure to these compounds, and 3) develop a method for measuring rumen motility in cattle for evaluation of the effect of ergot alkaloids on forestomach motility.

Copyright © Amanda M. Egert 2014

Chapter 2: Literature Review

Introduction

As a popular forage grass in the United States, beef cattle production enterprises relying on tall fescue consumption account for a large part of the beef industry and agricultural economy. Although the grass would appear to be a good forage for grazing animals, based on its nutrient composition and hardiness, the consumption of tall fescue infected with an endophytic fungus is associated with poor animal performance and negative health effects. The resulting syndrome, known as fescue toxicosis, is costly to producers. Research has focused on investigating beneficial properties the endophyte conveys to the grass, negative effects on mammalian systems, potential causative agents, and mechanisms by which the effects on animal performance are mediated. Despite a plethora of research, an animal solution to fescue toxicosis still remains elusive.

Tall Fescue

Tall fescue (*Lolium arundinaceum*, previously *Festuca arundinacea* Schreb.) is a common cool-season, perennial grass in the Pacific Northwest and Southeastern United States. A native of Europe, tall fescue is believed to have been a contaminant in meadow fescue seed, which was imported from England in the 1800s (Kennedy, 1900; Vinall, 1909). As early as 1900, grass trials in Kentucky (Garman, 1900) and Virginia (Kennedy, 1900) documented the superior qualities of tall fescue in comparison to meadow fescue, such as greater growth rate, height, competitiveness, and tolerance to drought.

When two tall fescue cultivars were released in the early 1940s, planting of tall fescue in the decades to follow created large stands in certain regions of the United

States (Hoveland, 2009). Alta was a cultivar released by the Oregon Agriculture Experiment Station and USDA and planted throughout the Pacific Northwest and regions between mountains of the west (Cowan, 1956). This cultivar was able to survive the harsh winters and provided green forage during the dry summers of these areas. Kentucky 31 (**KY-31**), the cultivar from which most tall fescue in the United States was grown, was collected from an eastern Kentucky mountain sheep pasture and released by the University of Kentucky in 1943 (Fergus, 1952; Fergus and Buckner, 1972; Rottinghaus et al., 1991). Kentucky-31 was particularly resilient, provided forage for grazing the majority of the year, and could be grown in a variety of soil types (Hoveland, 2009).

While older references state tall fescue covers approximately 14 to 15 million hectares (35 million acres) in the United States (Bacon and Siegel, 1988; Yates and Powell, 1988; Thompson et al., 2001), newer reports say tall fescue occupies more than 20 million hectares of pastureland (Parish et al., 2003; Bacetty et al., 2009). The predominant use of tall fescue has been for forage or pasture, but it is also used as a turf grass, conservation grass, and erosion control plant (Bacon and Siegel, 1988; Henson, 2001; Hoveland, 2009). Maps displaying suitable areas in the United States for tall fescue have been developed based on climate and soil factors (water supply, drainage, pH, and salinity; Hoveland, 2009).

Endophytic Symbiont

Epichloë coenophiala ((Morgan-Jones and Gams) Bacon and Schardl), a fungal endophyte, engages in a mutualistic symbiosis with tall fescue (Bacon et al., 1977; Leuchtmann et al., 2014). Initially, the endophyte was called *Epichloë typhina* (Pers.) Tul, but it was renamed *Acremonium coenphialum* (Morgan-Jones and Gams), changed to *Neotyphodium coenophialum* ((Morgan-Jones and Gams) Glenn, Bacon, and Hanlin),

and finally, classified as *Epichloë coenophialia* (Hoveland, 2009; Leuchtmann et al., 2014). Infection of tall fescue with this endophyte and its distribution was first documented by Sampson (1933). Living within the intercellular spaces of plant tissue, particularly the leaf sheaths and flowering stems, the endophyte causes a symptomless infection (Bacon and Siegel, 1988).

Concentrations of endophyte are highest within the leaf sheaths and seeds (Bacon and Siegel, 1988). Uninfected plants cannot become infected as it is strictly maternally or vertically transferred (Bacon and Siegel, 1988; Clay and Schardl, 2002; Schardl et al., 2004). Therefore, transmission of the endophyte occurs by seed (Siegel et al., 1984a; Bacon and Siegel, 1988). As a result, pastures that were once uninfected can become infected, not by invasion of the endophyte itself into already existing plants, but by the spreading of infected seed which will grow new infected plants. Over time, these infected plants will likely outcompete the uninfected plants and dominate the pasture.

The environment could play a role in levels of endophyte infection. Siegel et al. (1984a) found that after a severely hot, dry summer and cold winter, the infection level of seed decreased, but remained similar in the stem. On the other hand, McCulley et al. (2014) reported that increased temperatures (+ 3°C) for five years did not affect endophyte infection within tall fescue plants in a mixed species pasture. Additionally, storage procedures can alter the survival of viable endophyte in seed. Length of storage, temperature, and moisture content affect the survival of the fungus. Whereas cold, dry storage has been shown to preserve endophyte viability, prolonged storage at high temperatures decreases the viability (Latch and Christensen 1982; Siegel et al., 1984b).

Agronomic Qualities

As mentioned previously, *N. coenophialum* and tall fescue participate in mutualism, meaning both organisms benefit from the intimate association. While the tall fescue host plant provides nutrients, protection from the environment, and means of reproduction and dissemination to the endophyte, the fungus confers beneficial agronomic properties to the plant (Bacon and Siegel, 1988). Endophyte presence helps the plant survive when challenged with various abiotic and biotic stresses.

Several studies have demonstrated improved drought resistance of endophyte-infected tall fescue. When potted tall fescue plants were subjected to various levels of drought stress in greenhouse conditions, endophyte-infected plants were more productive and survived better compared to endophyte-free plants (Arechevaleta et al., 1989). Regardless of N fertilization level, 75% of the endophyte-free plants died, yet all of the endophyte-infected plants endured through the highest drought stress level. Additionally, endophyte-infected plants had 38% greater regrowth following harvest after low drought stress and 111% greater regrowth after high drought stress than endophyte-free counterparts. This study utilized a single genotype or clone of KY-31. However, not all clones likely behave the same, and thus, it was noted that several clones, such as would be present in a pasture situation, need to be studied (Arechevaleta et al., 1989; Bacon, 1993). Later, West et al. (1993) demonstrated field-grown endophyte-infected tall fescue survived better than endophyte-free plants following severe drought stress; endophyte-infected plants showed a smaller reduction in tiller density during the drought stress and a full recovery of tiller density after irrigation. Furthermore, Bouton et al. (1993a) concluded that summer drought played an integral role in the declining fitness and stand persistence of endophyte-free tall fescue compared to endophyte-infected.

Mechanisms related to improved drought tolerance and persistence of endophyte-infected compared to endophyte-free tall fescue cultivars could include increased root development (Richardson et al., 1990a), greater root length density (Carrow, 1996) and depth, increased leaf shedding (West et al., 1988, 1989) and rolling (Arechevaleta et al., 1989), lower stomatal conductances (Richardson et al., 1990b), and an improved ability for osmoregulation (Elmi et al., 1989).

Endophyte-infected tall fescue has been shown to more efficiently utilize nitrogen from the soil. Tillering has been shown to decrease in tall fescue with increasing drought stress (Arechevaleta et al., 1989). However, at the greatest N fertilization level, endophyte-infected plants displayed increased (36%) plant tillering compared to endophyte-free plants (Arechevaleta et al., 1989). Particularly at higher N fertilization levels, herbage yield was about 50% greater on endophyte-infected plants (Arechevaleta et al., 1989). This study only used one genotype of tall fescue and population effects may differ (Arechevaleta et al., 1989; Bacon, 1993). However, it did demonstrate that endophyte-infected tall fescue more efficiently utilizes nitrogen from the soil than endophyte-free. Lyons et al. (1990) discovered that endophyte-infected tall fescue plants had higher glutamine synthetase activity at low soil nitrogen levels compared to endophyte-free plants when looking at several genotypes. Increased activity of this enzyme, which aids in reassimilation of ammonia in plants, demonstrated again that endophyte-infected plants can use nitrogen more efficiently.

Association of *N. coenophialum* with tall fescue confers insect resistance to the plant. Generally, mycotoxins produced by the endophyte have been shown to protect against certain sap sucker species and stem miners, but do not deter root feeders or leaf grazers (Hoveland, 2009). While endophyte infection did not affect survival of fall armyworms (*Spodoptera frugiperda* (J. E. Smith)), it did affect larvae feeding preference

(Hardy et al., 1986; Ball et al., 2006), decrease weight gain (Clay et al., 1985), and accelerate development (Bultman and Conard, 1998). However, feeding was deterred (Johnson et al., 1985) and survival was decreased when several species of aphids were confined to endophyte-infected tall fescue plants. Species included the oat bird cherry aphid (*R. padi*; Eichenseer et al., 1991; Eichenseer and Dahlman, 1992), greenbug (*S. graminum*; Eichenseer and Dahlman, 1992), Russian wheat aphid (*D. noxia*; Kindler et al., 1991), and yellow sugarcane aphid (*S. flava*; Breen, 1993a). As a result, endophyte-infection helps protect tall fescue plants from insect herbivory and damage.

Similarly, tall fescue infected with the endophyte displays some pest resistant properties, such as parasitic nematode resistance. Field studies demonstrated reduced nematode numbers with endophyte infection (Pedersen and Rodriguez-Kabana, 1984; West et al., 1988). In a greenhouse study, Pedersen and colleagues (1988) found that endophyte-infected tall fescue plants were associated with less spiral (*Helicotylenchus* dihystera) and stubby root (*Paratrichodorus minor*) nematodes in the soil than endophyte-free plants. Likewise, root-knot nematode (*Meloidogyne marylandi*) numbers were almost reduced to zero in endophyte-infected tall fescue plant pots, while nematode numbers increased at least ten times in endophyte-free pots over a five week period (Elmi et al., 2000). *Pratylenchus scribneri*, a lesion nematode, was found to average three to seventeen nematodes per pot for endophyte-infected tall fescue pots versus 4,866 to 8,450 nematodes per pot for endophyte-free (Bacetty et al., 2009). Therefore, predation of roots by nematodes is decreased by endophyte infection of tall fescue, which may aid in the enhanced stand persistence and root growth during periods of drought stress.

The *N. coenophialum*-tall fescue symbiosis enhances the productivity and survival of the plant and endophyte, particularly through adverse situations such as

drought, predation, or soil nutrient deficiencies. Some of the same mechanisms that prevent predation of insects and nematodes also reduce mammalian herbivory and are associated with negative health effects in grazing animals, which creates problems for animal producers.

Fescue Toxicosis in Beef Cattle

Soon after the rapid planting of tall fescue in the 1940s and 1950s, producers noticed that animals grazing tall fescue displayed poor performance. The associated adverse effects on cattle grazing endophyte-infected tall fescue have been collectively termed fescue toxicosis syndrome (Schmidt et al., 1982). Although studies have shown other grazing species, such as sheep and horses, can experience issues related to endophyte-infected tall fescue consumption, this section will focus on cattle.

Disorders and Animal Performance

There are three disorders of beef cattle within fescue toxicosis: 1) summer slump, 2) fescue foot, and 3) fat necrosis. Additionally, females grazing endophyte-infected tall fescue demonstrate negative reproductive qualities.

Summer slump is the most common fescue toxicosis disorder of cattle and also contributes the greatest amount to associated production losses (Schmidt and Osborn, 1993). As a result, the term “fescue toxicosis” typically refers to this disorder. Generally, summer slump occurs at high environmental temperatures (Thompson and Stuedemann, 1993) during the spring and summer when cattle are heat stressed with the increased requirement for heat dissipation. Signs include increased salivation, higher respiratory rates, elevated rectal temperatures, reduced feed intake, decreased average daily gains, reduced time spent grazing, nervousness, and animal preference

for shade, mud holes, ponds, and other wet, cool locations (Bush et al., 1979; Hemken et al., 1984; Bond et al., 1984; Hoveland et al., 1983, 1993; Thompson and Stuedemann, 1993; Strickland et al., 2011). Endophyte-infected tall fescue palatability and increased body temperatures could act to decrease intake. Cattle will avoid eating an endophyte-infected tall fescue diet when given the option of an endophyte-free diet (Fribourg et al., 1991). Perhaps the most obvious sign of summer slump is retention of the winter hair coat in the spring resulting in a rough hair coat (Schmidt and Osborn, 1993). Many of the outward symptoms of summer slump suggest the cattle are unable to dissipate heat adequately. Overall, summer slump is characterized by poor growth performance and cattle appearance as well as changes in animal behavior.

The first documented reports of fescue foot were in 1949 (Australia), 1950 (New Zealand), and 1952 (United States; Yates, 1962). Fescue foot typically occurs during periods of cold environmental temperatures, particularly the winter (Garner and Cornell, 1978), and most often affects the hind feet (Thompson and Stuedemann, 1993). Associated symptoms begin with hyperemia (red color) of the hoof cornonary band, swelling of the fetlock, and soreness in the hind limbs. Cattle that are not removed from endophyte-infected tall fescue pastures can develop dry, gangrene on extremities, lameness, and eventually the dead tissues (hooves, ear tips, tail tips, or teats) may be sloughed in severe cases (Yates, 1962; Garner and Cornell, 1978; Bush et al., 1979; Schmidt and Osborn, 1993; Thompson and Stuedemann, 1993; Waller, 2009; Strickland et al., 2011). Normal thermoregulation would cause animals in colder temperatures to conserve body heat (Curtis, 1983). However, changes in blood flow to peripheral tissues (to be discussed later) associated with consumption of endophyte-infected tall fescue likely aid in the onset of fescue foot.

Endophyte-infected tall fescue pastures that are heavily fertilized with nitrogen are often associated with bovine fat necrosis, also known as lipomatosis, in grazing cattle (Wilkinson et al., 1983). Fat necrosis is a condition in which large masses of hard fat accumulate in the mesenteric adipose tissue along the gastrointestinal tract (**GIT**; Bush et al., 1979; Wilkinson et al., 1983). As a result, this necrotic fat takes up valuable space in the abdomen and can hinder the normal flow of digesta through the GIT. In extreme cases, strangulation of intestines may occur (Schmidt and Osborn, 1993). Additionally, reproductive issues, such as dystocia, could result due to interference with calving (Wilkinson et al., 1983; Thompson and Stuedemann, 1993). The relationship between high N fertilization and this disorder may be due to increased production of N-containing compounds associated with the etiology of the syndrome (to be discussed below; Lyons and Bacon, 1984; Lyons at al., 1986; Arechavaleta et al., 1992).

Many other negative reproductive effects have been associated with endophyte-infected tall fescue consumption. Significantly more research has been done on beef cows versus bulls because most tall fescue in the United States is consumed by cow-calf operations (Schmidt and Osborn, 1993). Studies have demonstrated cows consuming infected tall fescue show declines in body condition (Peters et al., 1992; Schmidt and Osborn, 1993), pregnancy rates by as much as 39.2% (Gay et al., 1988; Brown et al., 1992), calving rates by approximately 40% (Schmidt et al., 1986; Porter and Thompson, 1992; Hoveland, 1993), milk production (agalactia; Peters et al., 1992; Brown et al., 1993; Brown et al., 1996), and calf weaning weights (Peters et al., 1992; Schmidt and Osborn, 1993). Angus heifers grazing endophyte-infected tall fescue also experienced delayed puberty (Washburn et al., 1989). Recently, a study found that gestating ewes who consumed endpophyte-infected tall fescue seed had shorter gestation periods, lower lamb birth weights, and reduced fetal muscle development

(Duckett et al., 2014). This provided evidence of decreased growth and altered fetal development in utero with E+ consumption, but this study did not measure the direct exposure of the fetus to potential causative agents or feed intake of lambs, which could have affected growth.

One of the most important issues related to cattle productivity when grazing endophyte-infected tall fescue is reduced body weight gains. Steers fed diets containing 60% fungus-infested seed or 85% chopped fungus-infested hay displayed lower gains, decreased intake (36% decrease on seed; 8% decrease on chopped hay), and higher rectal temperatures (by 0.6°C) than their equivalent fungus-free diets over a 53 day period (Schmidt et al., 1982). Another study conducted in Alabama showed similar reductions in steer performance (Hoveland et al., 1983). In addition, non-infested pastures produced an average of 28% more beef gain per hectare than fungus-infested pastures. Likewise, Read and Camp (1986) found that over a three year grazing study using Kenhy tall fescue in Texas, ADG of steers were 0.46 kg on pastures highly infected with endophyte and 0.97 kg on pastures with a low endophyte level. Gain per hectare was also lower for steers on high-endophyte tall fescue (2.90 kg/ha/day versus 4.70 kg/ha/day). Crawford et al. (1989a) discovered a significant relationship between endophyte-infection frequency (**EIF**) and ADG of Holstein steers and heifers resulting in the following equation: ADG (kg/day) = 0.66 – 0.0045 × EIF(%). Particularly for the spring and summer periods, each 10% increase in EIF resulted in an approximate 0.07 kg/day reduction in animal gain. On the other hand, cattle who have previously grazed endophyte-infected tall fescue exhibit compensatory gain in the feedlot (Cole et al., 1987; McDonald et al., 1988; Lusby et al., 1990). Therefore, effects of endophyte exposure on gain are not permanent.

Consumption of endophyte-infected tall fescue alters the ability of cattle to regulate body temperature. The effects of endophyte-infected tall fescue consumption on difficulty dissipating heat (heat stress) are particularly exacerbated at high ambient temperatures and humidity (Schmidt and Osborn, 1993; Paterson et al., 1995), indicated by increased body temperatures, respiration rates, and salivation. Temperatures above 31°C resulted in decreases in intake and gain, as well as increases in rectal temperatures and respiration rates between cattle consuming endophyte-infected tall fescue (GI-307) compared to a less toxic variety (GI-306; Hemken et al., 1981). Similarly, Aldrich et al. (1993a) reported that steers consuming endophyte-infected tall fescue failed to show an increase in skin vaporization from steers housed at 22°C, whereas endophyte-free consuming steers had greater vaporization. The lack of vaporization increase for endophyte-infected consuming steers demonstrated a decreased ability to dissipate excess body heat. Neurotransmitter and endocrine imbalances from consumption of toxic substances in endophyte-infected tall fescue could affect the thermoregulatory center in the hypothalamus leading to the inability to regulate heat associated with fescue toxicosis (Marple et al., 1988; Schillo et al., 1988).

Low serum prolactin concentrations have become one of the best indicators of cattle experiencing fescue toxicosis. Reductions in serum prolactin have been consistently shown in cattle consuming endophyte-infected tall fescue (Hurley et al., 1980; Bolt et al., 1983; Thompson et al., 1987; Brown et al., 2009). Porter et al. (1985) reported decreased serum prolactin in rats given an extract of endophyte-infected seed. Decreased concentrations of prolactin in the pituitary gland of cattle have also been demonstrated (Schillo et al., 1988), yet this was not the case in ewes (Bolt et al., 1982). A short period of consuming endophyte-infected tall fescue can result in serum prolactin reductions, and recovery to normal levels is relatively quick after removal of endophyte-

infected tall fescue from the diet (Bolt et al., 1982). Prolactin is a protein hormone which affects milk production and mammogenesis. Reductions in prolactin in cows on endophyte-infected fescue could lead to the observed decreases in milk yield (Karg and Schams, 1974; Thompson and Stuedemann, 1993). A closer look at the mechanism by which the endophyte effects prolactin secretion will be presented in a later section.

Economic Impact

Due to the expansive amount of land occupied by tall fescue in the United States and high incidence of infection, fescue toxicosis is the greatest animal health-related production loss of the grazing industry (Strickland et al., 2011). Hoveland (1993) conducted surveys of extension specialists in twenty-one states and estimated reduced calving rates and weaning weights accounted for approximately $354 million and $255 million annual losses for the beef cattle industry, respectively. Combining these losses with decreased productivity of steers grazing endophyte-infected tall fescue and issues in other species of grazing livestock, a more accurate estimation of the annual loss is likely $1 billion (Strickland et al., 2011). Therefore, research has focused on investigating possible causative agent(s) of fescue toxicosis and methods of managing and alleviating the negative effects of endophyte-infected tall fescue.

Management Strategies

Since endophyte-infected tall fescue is more tolerant to drought and overgrazing, cattle producers have been reluctant to reestablish pastures with less persistent endophyte-free or low-endophyte varieties as this is an expensive and time consuming process (Hoveland, 1993; Gunter and Beck, 2004). As a result, several pasture and animal management methods have been tested in an attempt to alleviate the effects of fescus toxicosis in cattle grazing endophyte-infected tall fescue.

Pasture management strategies have focused on reducing endophyte consumption. The endophyte and toxic compounds are highly concentrated in the seed heads, so reducing seed head formation could help reduce the signs of fescue toxicosis (Schmidt and Osborn, 1993; Paterson et al., 1995). Methods include increased continuous grazing pressure (overstocking; Bransby et al., 1988), clipping, and use of chemical plant growth regulators such as mefluidide (Turner et al., 1990; Schmidt and Osborn, 1993; Paterson et al., 1995). Animals will selectively graze seed heads at lower stocking rates, so by increasing stocking rates, seed head formation will be reduced. Goff (2012) demonstrated that reducing seed heads by chemical means could increase ADG by 64%. Additionally, interseeding of endophyte-infected pastures with legumes (e.g. clovers or birdsfoot trefoil) works to dilute the toxins and thereby, help alleviate some symptoms of fescue toxicosis in steers and cow-calves (Hoveland et al., 1981; Ellis et al., 1983; McMurphy et al., 1990; Chestnut et al., 1991; Gay et al., 1988; Waller et al., 1989). Despite some improvements, the application of these techniques has not provided overwhelming success.

Other animal management practices, along with the previously mentioned overstocking, have been studied in an attempt to find approaches to help minimize effects of fescue toxicosis. For example, removing cattle from endophyte-infected pastures to non-fescue, warm-season annual pastures during periods of high ambient temperatures, such as July, can increase ADG (Aldrich et al., 1990; Forcherio et al., 1992) and weaning weights (Rhodes et al., 1991) of calves to levels of non-endophyte exposed animals. Likewise, creep grazing or providing a high quality forage to calves, particularly when forage quality and milk production decline, can increase calf gain on endophyte-infected tall fescue pastures to the level of calves on endophyte-free pastures without creep grazing (Tucker et al., 1989). Supplementation of nutrients to cattle has

also been shown to be beneficial to cattle grazing endophyte-infected tall fescue. Addition of an 85% corn: 15% soybean meal mixture (0.9 kg/d) to growing steers consuming tall fescue increased ADG (Crawford et al., 1989b). Similarly, Elizadale et al. (1998) found that supplementation of spring grazing Angus steers with various levels of protein and energy through corn and corn byproducts improved ADG over controls yet did not affect finishing phase performance, ruminal fermentation, OM digestibility, or net microbial protein synthesis in the rumen. However, the effects of energy and protein supplementation cannot be separated in this study. Another study found that addition of blood meal, a form of ruminally undegradable protein, to young lactating beef cows grazing endophyte-infected tall fescue in spring and early summer increased weight gain of cows (Forcherio et al., 1993). Growth stimulants or implants, anthelmintics (ivermectin), aluminosilicates, selenium injections, and other treatments have all been considered for alleviating fescue toxicosis (Schmidt and Osborn, 1993; Paterson et al., 1995). Nevertheless, controlled research of these treatment methods has resulted in little success.

A promising area of research is related to genetic alteration of the endophyte to produce novel or non-toxic varieties. Ideally, these novel endophytes would still provide the beneficial agronomic qualities to the tall fescue plant, but would not produce the toxins which cause fescue toxicosis, assuming that the compounds creating these effects are different (Bacon and Siegel, 1988; Hoveland et al., 1993). Examples of novel endophyte-infected tall fescues that have been created include Jesup AR542 (Bouton et al., 1997), Georgia 5 AR542 (Bouton et al., 1993b), and HiMag Strain 4 (Sleper et al., 2002). Several studies have tested the novel endophyte cultivars for their effect on plant fitness and animal performance. Bouton et al. (2002) found that after two years of grazing by sheep, Jesup AR542 was as persistent as wild-type endophyte-infected tall

fescue. Lambs showed no signs of fescue toxicosis and had greater ADG when grazing the novel endophyte (AR542) in either Jesup or Georgia 5 tall fescue. However, lambs on wild-type infected Jesup displayed higher rectal temperatures and decreased serum prolactin. Similarly, Parish et al. (2003) and Nihsen et al. (2004) demonstrated enhanced cattle productivity on novel endophyte tall fescue compared to the wild-type endophyte-infected tall fescue as evidenced by greater ADG, higher DM intakes, lower temperatures, slower respiration rates, better hair coats, and increased prolactin concentrations. On the other hand, replanting and reestablishing wild-type endophyte-infected pastures with novel endophyte-infected pastures is an expensive and time consuming process that Gunter and Beck (2004) estimated would take three years to pay back and begin receiving profit. Thus, the use of novel endophytes for alleviating fescue toxicosis is not widely used throughout the beef cattle industry in the United States.

What Causes Fescue Toxicosis?

Numerous factors are involved in the complex syndrome of fescue toxicosis, and the mechanism is still not fully understood today. Reduced forage intake and decreased blood flow could account for many of the signs and production losses associated with fescue toxicosis. Thus, antiherbivory and vasoactive compounds have been most heavily investigated as possible causative agents.

In the 1950s, post mortem analysis of cattle experiencing lameness while consuming tall fescue hay uncovered thrombosis of the arteries which obstructed blood flow (Jensen et al., 1955). As a result, researchers concluded that a toxic substance in tall fescue causes vasoconstriction in peripheral tissues. It was believed that this could be the cause of gangrene associated with fescue foot. However, the specific compounds signaling the vasoconstriction were still unknown. Symptoms of fescue

toxicosis, specifically fescue foot, appeared very similar to ergotism from ergot sclerotia of *Claviceps purpurae* fungus (Maag and Tobiska, 1956). Yet, ergot sclerotia were not found on tall fescue plants leading to the dismissal of *C. purpurae* involvement in fescue toxicity (Maag and Tobiska, 1956; Cunningham, 1949).

When Bacon and colleagues (1977) isolated the endophytic fungus *Epichloë typhina* from tall fescue, they hypothesized that mycotoxins produced by the endophyte could be responsible for fescue toxicosis related animal health and productivity problems. Many fungal endophyte-grass symbioses were known to produce various bioprotective compounds or alkaloids. As a result, research has been aimed at the four groups of alkaloids associated with *N. coenophialum* infection of tall fescue: diazaphenanthrenes, pyrrolopyrazines, pyrrolizidines, and ergot alkaloids (Bush and Burrus, 1988; Siegel et al., 1990; Bush et al., 1997).

Perloline, the main diazaphenanthrene alkaloid, is produced by the tall fescue plant, rather than the endophyte (Bush and Burrus, 1988). Boling et al. (1975) found perloline increased body temperatures and decreased apparent cellulose digestibility in sheep. However, studies reported perloline was present in endophyte-free varieties, and the presence of endophyte in tall fescue did not affect perloline concentration (Ribeiro and Bush, 1983; Strahan et al., 1987). The environment and plant genetics can impact the perloline concentration of tall fescue (Buckner et al., 1973; Bush et al., 1979). Due to the lack of association between perloline content, endophyte presence, and links to signs of fescue toxicosis, perloline is likely not one of the causative agents.

Peramine, the only known pyrrolopyrazine alkaloid isolated from tall fescue, functions as an insect deterent and is the most common grass-fungal associated alkaloid

(Bush and Fannin, 2009). However, mammalian bioassays resulted in the conclusion that peramine was not bioactive in mammals (Bush and Fannin, 2009).

The first chemical differences between infected and uninfected tall fescue were found in N-formylloline and N-acetylloline of the pyrrolizidine group (Bush et al., 1982; Jones et al., 1983). With insecticidal activity, N-formylloline has been shown to act as an insect feeding deterrent and metabolic toxin (Johnson et al., 1985; Dahlman et al., 1997; Bush et al., 1997). Some bioactivity in mammals has been demonstrated but to a lesser extent than ergot alkaloids. Klotz et al. (2008) found no response of lateral saphenous veins in vitro to N-acetyl loline. Numerous investigations have shown endophyte-free tall fescue seed or forage had little or no loline content whereas endophyte-infected had at least 550 ppm (Neil and Schmidt, 1985; Strahan et al., 1987). Additionally, Jackson et al. (1984) fed Holstein calves a high loline (577 µg N-acetyl plus N-formyl loline alkaloids per g) diet of Kenhy tall fescue seed, low loline (0 µg/g) diet of Kenhy tall fescue seed, or a control diet (0 µg/g) of mostly cracked corn and rolled oats at high environmental temperatures (31-32°C). Results indicated that the high loline diet produced signs of fescue toxicosis, such as increased rectal temperatures, decreased feed intake, and body weight loss, whereas the diet without lolines did not. A similar pattern was seen with hay. However, the other types of alkaloid that may have been present were not measured in this study. Therefore, one cannot solely attribute these fescue toxicosis symptoms to the loline alkaloids when perhaps endophyte infestation level could be responsible (Foote, 2013).

Ergot alkaloids, the final class of compounds associated with tall fescue-endophyte symbiosis, are mycotoxins produced by several fungal species in vitro and within infected plants (Porter et al., 1979; Lyons and Bacon, 1984; Bacon, 1988; Strickland et al., 2011). Clavines, lysergic acid and its derivatives (water-soluble), and

ergopeptine (water-insoluble) alkaloids make up the three groups of ergot alkaloids. As much as 50% of the total alkaloids present are ergopeptines (Lyons et al., 1986). Production levels of ergopeptide alkaloids in endophyte-infected tall fescue are increased with drought stress (Arechavaleta et al., 1992), environmental warming, (McCulley et al., 2014), and N-fertilization of plants (Lyons and Bacon, 1984; Lyons at al., 1986; Arechavaleta et al., 1992). Ergovaline is the most abundant ergot alkaloid in tall fescue, which comprises the 84 to 97% of the ergopeptide alkaloids present and has been shown to be toxic (Lyons et al., 1986; Bush et al., 1997). Since ergovaline has not been detected in a measurable quantity in *C. purpurea*, the dismissal of this endophyte's involvement in fescue toxicosis was warranted (Belesky et al., 1988).

Although research has not been conclusive in implicating a single alkaloid as the compound responsible for inducing fescue toxicosis in grazing cattle, ergot alkaloids, most commonly ergovaline, are proposed to be the primary causative agents. A more detailed description of this group of alkaloids is provided below.

Ergot Alkaloids

The association of this group of compounds with fescue toxicosis has been related to their activity within mammalian systems. Toxic tall fescue contains ergopeptides, lysergic acid, and lysergic acid derivatives. Lysergic acid and its derivatives are commonly referred to as ergolines. Bush and Burrus (1988) complied results from multiple studies and determined that the average total ergot alkaloid content of endophyte-infected KY-31 was 0.95 ppm, whereas endophyte free KY-31 had no ergot alkaloids. Even though the content of the loline alkaloids (approximately 2500 ppm; Bush and Burrus, 1988) greatly exceeds that of ergot alkaloids and one could

presume that lolines are therefore the causative agent of fescue toxicosis, the lolines are much less bioactive in mammalian systems than ergot alkaloids (Bush et al., 1997). While ergovaline is the predominant ergot alkaloid studied, additive effects of numerous alkaloids may be present that contribute to the effects of fescue toxicosis (Klotz et al., 2008).

Structure, Synthesis, and Modes of Action

A tetracyclic ergoline ring structure is the defining characteristic of ergot alkaloids (Berde, 1980; Weber, 1980). Synthesis of ergot alkaloids begins with combining dimethylallyl diphosphate (DMAPP) and L-tryptophan to form dimethylallyl-tryptophan (Bush et al., 1997). This reaction is catalyzed by DMAT synthase and is likely the rate-limiting step. Conversion of DMAT to lysergic acid and eventually its derivatives and ergopeptides then follows (Bush et al., 1997) Lysergic acid and derivatives thereof lack the cyclic tripeptide moiety attached to the ergoline ring, which is included in the structure of ergopetides (Bush and Fannin, 2009; Strickland et al., 2011). While all ergopeptides have proline at the third amino acid position, eropeptides vary at two positions of the cyclic tripeptide (Bush and Fannin, 2009).

The biological activity and physiological effects of ergot alkaloids are enabled by their structure and properties. The ergoline ring system resembles that of biogenic amine neurotransmitters, such as norepinephrine, epinephrine, dopamine, and serotonin, allowing ergot alkaloids to bind their receptors and cause a response (Strickland et al., 2011). Floss and colleagues (1973) discovered that dopaminergic, serotonergic, and α-adrenergic receptors were stimulated by ergot alkaloids, which affected thermoregulatory and vasoregulatory pathways. These receptors and their associated neurotransmitters help regulate cardiovascular function, endocrine activity, motility of the gastrointestinal tract, smooth muscle contraction, temperature regulation,

and appetite. Thus, the action of ergot alkaloids on biogenic amine receptors could result in many of the symptoms of fescue toxicosis by alteration of these regulatory pathways.

Binding of ergot alkaloids to D2-dopamine receptors stimulates the release of dopamine which inhibits prolactin release (Berde and Stürmer, 1978; Goldstein et al., 1980; Sibley and Creese, 1983; Schillo et al., 1988). Therefore, stimulation of D2 receptors is the mechanism by which animals consuming endophyte-infected tall fescue display decreases in circulating prolactin (Goetsch et al., 1987). Larson et al. (1995, 1999) found that the binding affinities of ergopeptide and ergoline alkaloids as agonists for the D2 receptors were similar to that of dopamine itself. Vasoactive intestinal peptide (VIP)-stimulated cyclic AMP production in GH_4ZR_7 cells was inhibited by ergovaline, ergonovine, ergotamine, and ergocryptine (Larson et al., 1995). Additionally, Maurer (1981) found that ergot alkaloids could also alter prolactin gene expression. Some studies have indicated that dopamine antagonists, such as metoclopramide and phenothiazine, can increase prolactin levels and ADG in cattle on endophyte-infected tall fescue, but show little improvements in other signs of fescue toxicosis (Bolt et al., 1983; Boling et al., 1989; Lipham et al., 1989).

Various experimental approaches have been used to detect and quantify ergot alkaloids including enzyme-linked immunosorbent assay (**ELISA**; Shelby and Kelley, 1990, 1992; Schnitzius et al., 2001), high-performance liquid chromatography (**HPLC**) coupled to either fluorescence detection (Yates and Powell, 1988; Rottinghaus et al., 1991) or mass spectrometry (**MS**; Yates et al., 1985; Smith et al., 2009). While the ELISA method allows a quick determination of total ergot alkaloids, the HPLC-fluorescence method increases the selectivity by distinguishing individual ergot alkaloids (Strickland et al., 2011). Furthermore, HPLC-MS and HPLC-MS/MS deduce a higher

level of compound identification and can even help identify unknown compounds (Strickland et al., 2011).

Metabolism and Absorption

Cattle are pregastric ruminant fermenters based on their digestive physiology, which means that after ingestion, feed is subjected to fermentation by microbes (bacteria, protozoa, and fungi) in the forestomach before acid digestion in the abomasum (Church, 1976). Therefore, ergot alkaloids consumed in the diet have an opportunity to be metabolized, digested, degraded, and detoxified by microbial processes before mammalian enzymes interact with these compounds.

It is believed that the majority of alkaloid metabolism takes place in the rumen (Westendorf et al., 1992; Hill et al., 2001). Incubation of endophyte-infected KY-31 seed for twenty-four hours with rumen fluid decreased the toxicity in Harlan Sprague-Dawley rats suggesting that microbial fermentation may detoxify or degrade compounds responsible for fescue toxicosis (Westendorf et al., 1992). Total loline alkaloid content actually increased with incubation (2540 vs. 2680 µg/g), which could indicate that ergot alkaloids might have been converted to these less active forms. Other studies have demonstrated that ergot alkaloids may be released from plant tissues as a result of microbial fermentation. When endophyte-infected tall fescue was inoculated with viable microbes in rumen fluid, the concentration of total ergot alkaloids increased in vitro as compared to inoculation with autoclaved rumen fluid (Ayers et al., 2009). Lysergic acid concentrations were also increased following 48 hours of inoculation. Additionally, De Lorme et al. (2007) reported that concentrations of ergovaline in rumen fluid increased at each time point sampled (d 0, 3, and 28) in wethers consuming endophyte-infected tall fescue straw. Ruminal fluid concentrations of lysergic acid increased from d 0 to 3 but were not different from d 3 to 28. While only 35% of ergovaline in the diet was

accounted for in the urine and feces, 248% of lysergic acid was recovered. Low ergovaline excretion could be due to conversion to lysergic acid or other compounds, but may also be due to accumulation in blood vessels (Klotz et al., 2009) or subcutaneous fat (Realini et al., 2005). Together, these studies suggest that ergot alkaloids are liberated by microbial fermentation of forages, and lysergic acid may be the excretory product of ergot alkaloid breakdown. Absorption sites for ergot alkaloids are proposed throughout the GIT with the primary location suggested to be the forestomach (Westendorf et al., 1992; Hill et al., 2001).

After absorption, ergot alkaloids may be excreted from the body through the feces, urine, or bile (Stuedemann et al., 1998; Schultz et al., 2006). Westendorf et al. (1993) found that abomasal contents contained only 50 to 60% of ergot alkaloids in the diet and fecal recovery was only 5% for sheep. Total GIT absorption was reported to be 93 to 97%. Therefore, approximately 40 to 50% of alkaloids are absorbed or metabolized in the forestomach. This study supported results of Stuedemann et al. (1998) who measured ergot alkaloid excretion in urine and bile and found that urinary excretion accounted for about 95% of the total ergot alkaloids consumed by steers grazing endophyte-infected tall fescue.

Although there have been no in vivo studies of specific ergot alkaloid absorption and transport mechanisms, several in vitro studies have provided evidence of ergot alkaloid absorption. After four hour incubations of several ergot alkaloids at equivalent concentrations in a parabiotic chamber, Hill et al. (2001) showed that ergot alkaloids (lysergic acid, lysergol, ergonovine, ergotamine, and ergocryptine) can cross ruminal, reticular, and omasal tissues isolated from sheep. Yet, lysergic acid concentrations on the serosal side were more than 50% greater than other ergot alkaloids. Omasal tissues showed more absorption potential of ergopeptides than ruminal tissues, which is likely

related to their greater surface area, and ruminal tissues displayed 600% the absorption potential as reticular tissues. It is important to recognize here that in endophyte-infected tall fescue, ergot alkaloids are not present in equal concentrations which could affect rate of transport. An indication of the type of transport involved in ergot alkaloid absorption has been provided by this study; sodium azide, which prevents active transport and cellular metabolism essentially killing the tissues, inhibited ergot alkaloid movement across the epithelium after 240 min of incubation, suggesting active transport mechanisms of absorption (Hill et al., 2001). While Ayers et al. (2009) found ergovaline did not cross sheep ruminal or omasal mucosa, Shappell and Smith (2005) demonstrated that ergovaline was transported through Caco-2 cells (a line of human intestinal cells). Foote (2013) also showed that ergovaline flux occurred across naïve ruminal epithelium using an Ussing chamber. Further investigation of ergot alkaloids metabolism and absorption, including identification of specific mechanisms, is needed.

Vasoactivity and Associated Mechanisms

Ergot alkaloids have been shown to cause vasoconstriction of bovine core (duodenum and colon, Rhodes et al., 1991; uterine and umbilical arteries, Dyer, 1993), peripheral (dorsal pedal vein, Solomons et al., 1989; lateral saphenous vein, Klotz et al., 2006; caudal artery, Aiken et al., 2007), and foregut (right ruminal artery and vein, Foote et al., 2011) blood vessels and decrease blood flow to skin over the ribs, cerebellum (Rhodes et al., 1991), and reticulorumen epithelium (Foote et al., 2013). As mentioned previously, many of the symptoms of fescue toxicosis caused by endophyte-infected tall fescue consumption could be due largely to peripheral vasoconstriction. Lateral saphenous veins of steers grazing high endophyte-infected tall fescue pastures had smaller inner diameters compared to those on low-endophyte mixed pastures (Klotz et al., 2012). This would result in decreased blood flow to peripheral tissues and skin and

could lead to a decreased ability for evaporative heat loss in hot temperatures and hypothermia or gangrene of the extremities in cold temperatures.

Ergopeptide alkaloids are potent vasoconstriction agents. Several in vitro assays have provided a way of measuring and comparing the vasocontractility of different ergot alkaloids. Dyer (1993) provided evidence that ergovaline, the most abundant ergopeptide, was 75-times more potent than serotonin (5HT) at inducing vasoconstriction of the cow uterine artery. Klotz et al. (2006, 2007) found ergovaline produced a much larger vasoconstriction response in the lateral saphenous vein compared to lysergic acid. Additionally, Foote et al. (2011, 2012) demonstrated that ergovaline had the lowest EC_{50} (indicating high potency) and highest E_{max} (maximum contraction response or efficacy) compared to ergonovine, ergocornine, ergocryptine, ergocristine, and ergotamine in right ruminal artery (2011) as well as an ergot alkaloid mixture and endophyte-infected tall fescue seed extract in lateral saphenous vein (2012). Yet, ergonovine, a lysergic acid derivative, was shown to have similar vasoconstrictive potential as ergocornine, an ergopeptide alkaloid (Klotz et al., 2010).

Documented mechanisms by which ergot alkaloids elicit vasoconstriction are not fully understood, sometimes contradictory, and it is likely that a multitude of mechanisms contribute. Use of a $5HT_{2A}$ receptor antagonist, ketanserin, reduced vasoconstriction of bovine uterine and fetal umbilical arteries induced by ergovaline in vitro, but compounds specific to α-adrenergic receptors did not (Dyer, 1993). As a result, this researcher suggested that 5HT receptor activation by ergot alkaloids in endophyte-infected tall fescue was the primary cause of its toxic effects. Serotonin interacts with the satiety center in the brain and thus, ergot alkaloids acting via this mechanism could also cause a reduction in feed intake. Another study using rat tail arteries showed that vasoconstriction can be induced by ergotamine and ergovaline through $5HT_{2A}$, $5HT_{1B/1D}$,

and α_1-adrenergic receptors (Schöning et al, 2001). Additionally, ergovaline appeared to have both agonist and antagonist effects on the 5HT1B/1D receptor. In 2012, Klotz et al. investigated several different serotonin receptor type agonists and concluded that $5HT_{2A}$ and $5HT_7$ subtypes were the receptors primarily involved in vasoconstriction associated with fescue toxicosis. Some evidence has also been provided to suggest that α_2-adrenergic receptors could be involved (Oliver et al., 1998).

The specific compound, tissue type, and immediate prior exposure to endophyte-infected tall fescue can greatly affect the vasoactivity. This may be why the responses of vasculature to ergot alkaloids in the literature varies. Oliver et al. (1993), for example, demonstrated that the dorsal metatarsal artery and lateral saphenous vein responded differently to lysergamide in an in vitro myograph assay. Furthermore, lateral saphenous veins collected from steers grazing high-endophyte tall fescue had smaller contractile responses to ergovaline and serotonin than steers grazing low-endophyte pasture (Klotz et al., 2012). This study suggested that previous exposure to ergot alkaloids could affect the vasoactivity of ergot alkaloids in vitro. Ergot alkaloids may act as agonists, partial agonists, antagonists, or partial antagonists, which could help explain why responses to ergot alkaloids are varied and complex (Berde, 1980).

Although the mechanisms of vasoconstriction may not be fully understood, there is no denying the relationship between ergot alkaloid induced vasoconstriction and signs of fescue toxicosis. Determination of specific receptors by which ergot alkaloids elicit a response could aid in pharmacological or therapeutic approaches for alleviating fescue toxicosis in beef cattle.

Effect on the Gastrointestinal Tract

Alterations in the function of the gastrointestinal tract could pose serious threats to the health and performance of beef cattle. Since ergot alkaloids ingested with endophyte-infected tall fescue enter the gastrointestinal tract, the toxic effects of these compounds could affect nutrient digestion and absorption. Several studies testing for differences in digestibility between endophyte-infected and endophyte-free tall fescue have generated a variety of results, yet it is difficult to compare studies with different forage sources, endophyte species, and alkaloid concentrations. Relatively few studies have investigated other effects of ergot alkaloids on the gastrointestinal tracts of grazing livestock.

No direct evidence has been found to suggest that the ergot alkaloid-containing endophyte-infected tall fescue consumption affects the microbial community or ruminal fermentation. Methane production, ruminal pH, and ruminal ammonia were not affected by consumption of ergot alkaloids in endophyte-infected tall fescue (Aldrich et al., 1993b; Pavao-Zuckerman et al., 1999; De Lorme et al., 2007). On the other hand, antimicrobial activity has been reported (Looper et al., 2008).

Splanchnic blood flow is necessary for the function of the gastrointestinal tract (Matheson et al., 2000). As nutrients move across the mucosa, arterial blood flow typically increases to aid in the absorption of nutrients (Chou et al., 1992). Rhodes et al. (1991) determined that duodenal and colonic blood flow were decreased in cattle consuming endophyte-infected tall fescue. Similarly, Foote et al. (2013) observed a reduction in blood flow to the reticuloruminal epithelium with an endophyte-infected seed treatment. In contrast, portal vein blood flow was not affected by endophyte-infected tall fescue consumption (Harmon et al., 1991). Vasoconstriction and reductions in blood flow caused by ergot alkaloids in endophyte-infected tall fescue could negatively impact

nutrient absorption from the gastrointestinal tract. Decreased nutrient uptake may account in part for the reduced growth performance in cattle grazing endophyte-infected tall fescue. Only two studies have measured absorption of nutrients by cattle consuming endophtye-infected tall fescue or seed but similar effects were seen. Net portal acetate flux (Harmon et al., 1991) and absorption of volatile fatty acids from the reticulorumen (Foote et al., 2013) were decreased in cattle consuming the endophyte. Further work is needed to determine the effect of ergot alkaloids on intestinal vasculature and nutrient absorption.

Motility of the gastrointestinal tract and contractions of the forestomach are regulated by biogenic amine receptors and therefore, may also be affected by ergot alkaloid consumption. Although an effect of intraruminally or orally dosed ergot alkaloids on ruminant forestomach motility has not been reported, intravenous injection has been studied using electromyography (McLeay and Smith, 2006; Poole et al., 2009). When ergotamine and ergovaline were injected separately and intravenously, baseline tonus of reticulorumen smooth muscle was increased and cyclic A and B contractions were inhibited in sheep (McLeay and Smith, 2006). Similarly, Poole et al. (2009) demonstrated injecting ergotamine intravenously in sheep decreased reticular contraction frequency and increased baseline. In addition to determining the influence of ruminal and oral administration of ergot alkaloids on gastrointestinal tract motility, studies must be conducted in the bovine model along with the other sections of the gastrointestinal tract as well. A more detailed review of forestomach motility is provided in the following section.

Ruminant Forestomach Motility

Anatomy and development of the ruminant forestomach affects feeding behavior, type of feed consumed, absorption of nutrients, and motility. The importance of forestomach motility relates to its ability to influence rumination, removal of gasses (eructation), fluid and particulate passage rates, and digestibility of forages. Therefore, it is important to understand the basic anatomy and physiology of the forestomach and other GIT components to understand how and why it functions as it does.

The stomach of true ruminants, such as cattle, is quadrolocular (composed of four compartments): the rumen, reticulum, omasum, and abomasum (Church, 1976). Epithelium of the forestomach (rumen, reticulum, and omasum) is a non-glandular, cornified, stratified squamous tissue (Lavker et al., 1969). Because the abomasum consists of glandular, columnar cells, it functions similar to the stomach of nonruminants in which digestion occurs via acids and enzymes (Church, 1976). The rumen contains five sacs separated by pillars: the dorsal sac, caudo-dorsal sac, caudo-ventral sac, ventral sac, and crainial sac. This is the largest compartment and is the primary site for microbial fermentation of feedstuffs, which allows ruminants to more efficiently utilize structural carbohydrates. Papillae of the rumen are larger in the cranial and ventral sacs, thereby increasing the surface area and absorptive capacity. The reticulum has a characteristic honeycomb cell structure and is separated from the rumen by the reticulo-ruminal fold. The omasum contains laminae or leaves of tissue which provide about one third of the total forestomach surface area in cattle (Stevens and Stettler, 1966). Presence of a well-developed omasum in cattle indicates a greater ability to utilize fiber and likely helps filter the passage of particles (Van Soest, 1982). The cardia, or end of the esophagus, is located above the reticular groove, which closes and enables milk to bypass microbial fermentation in the rumen and directs it through the reticulo-omasal

orifice into the abomasum of young neonates (Church, 1976). In adult cattle, the rumen, reticulum, omasum, and abomasum constitute approximately 50-55%, 5-7%, 26-30%, and 13-14% of the total wet stomach tissues, respectively.

Movement Patterns of the Reticulorumen

Most of the research characterizing ruminant forestomach motility was conducted in the early to mid-1900s. Recent research even attempting to perturb the normal sequence of contractions is deficient. Contractions of the rumen and reticulum function to mix ingesta, eructate gas, regurgitate a bolus, and pass food onto the omasum when appropriately digested by microbial fermentation (Church, 1976). Patterns of reticuloruminal contractions result mainly from contractions of the pillars within the rumen. Contractions are cyclical and consist of two main types: primary (A or mixing cycles) and secondary (B or eructation cycles).

Primary sequences are initiated by a strong contraction of the reticulum, to about 50% of its resting size, and the reticulo-ruminal fold (Church, 1976; Sellers and Stevens, 1966). Then, a second, more powerful contraction of the reticulum occurs. Next, the reticulum relaxes, and a gradual wave of contractions and relaxations moves caudally. The crainial pillars contract and lift the cranial sac, caudal and dorsal coronary pillars and longitudinal pillars contract, which compresses the dorsal sac. Lastly, the caudo-dorsal blind sac, ventral coronary pillar, caudo-ventral blind sac, and ventral sac contract, completing the cycle. As a result, digesta particles in the mat are mixed with liquid digesta in what appears to be a figure eight shape.

Secondary sequences, initiated by the caudo-ventral blind sac, show a circular pattern and may occur by themselves or after a primary contraction (Ruckebusch and Tomov, 1973). Contractions proceed through the dorsal coronary pillar, caudo-dorsal

blind sac, and dorsal sac, followed by relaxation of the caudo-ventral blind sac. However, the cranial and longitudinal pillars may be involved as well, with the cranial pillar staying partially contracted to block digesta from returning to the reticulum (Reid and Cornwall, 1959). The contraction of the dorsal sac causes eructation of gas, although eructation does not always occur (Church, 1976). Without this process to allow the gases produced by microbial fermenetation (CO_2 and methane) to escape, cattle would experience bloat, which could cause death in severe cases.

Rumination is the process of regurgitating ingesta, remastication, reinsalivation, and redeglutition stimulated by mechanical stimulation of epithelial receptors within the reticulorumen (Church, 1976). Regurgitation of reticular contents typically occurs before the primary biphasic reticular contraction and is related to a solitary reticular contraction which increases pressure at the cardia but is not part of the primary or secondary cycles.

Factors that Affect Reticuloruminal Motility

Although primary and secondary sequences are the most often observed patterns of reticulorumen motility, variation among motility patterns exists (Church, 1976). These may be due to differences in the methods used to observe them or the multitude of factors that influence forestomach motility, such as species differences, diet composition, DM and water intake, ambient temperature, activity, cannulation, volatile fatty acid concentrations, metabolic conditions, and normal animal variation.

Cattle display differences in reticuloruminal motility from other ruminant species such as, sheep, goats, and buffalo, and greater variation between animals (Dziuk and McCauley, 1965). Relaxation of the reticulum after the first contraction in the primary sequence is almost complete creating two separate events in cattle, whereas that of goats and sheep is biphasic. McSweeney et al. (1989) found that water buffalo (*Bubalus*

bubalis) had fewer A and B contraction sequences than cattle (*Bos indicus*). Additionally, the ratio of A to B sequence contractions remained similar throughout a twenty-four hour period for mature cows, yet sheep displayed an increased ratio throughout the day due to a reduction in B sequence frequency (Waghorn and Reid, 1983).

Effects of other factors previously mentioned have been found. For example, eating versus resting activity greatly affects reticuloruminal motility. Cattle were shown to increase contraction rate with ingestion of food from 60 to 105 cycles per hour (Schalk and Amadon, 1928; Balch et al. 1951). Conversely, contractions become weak and slow with fasting or water deprivation (Attebery and Johnson, 1969; Schalk and Amadon, 1928). Cows exposed to environmental temperatures up to 38°C for up to 5 days displayed a reduction in contraction amplitude and a tendency for a decline in contraction frequency (Attebery and Johnson, 1969). Ash (1959), Bruce and Huber (1973), and Gregory (1984b) demonstrated that addition of short-chain volatile fatty acids to the rumen, abomasum, and intestines decreased contraction amplitude and frequency.

Perhaps one of the reasons for variation in results of retriculorumen motility lies in the numerous methods that have been used to measure contractions or motility. Exteriorizations, observation from open rumen fistula (Schalk and Amadon, 1928), implanted balloons, strain gauges, radiography, electromyography, radiotelemetry (Dziuk, 1964), and pressure-sensitive recordings of ruminal gas (Colvin and Daniels, 1965) or fluids (Dado and Allen, 1993) have all been utilized to measure and understand ruminant forestomach motility (Wyburn, 1980). The inconsistency of methods has appeared to result in some discrepancies in the data. Therefore, an accurate, consistent

method needs to be developed for measuring reticuloruminal motility for research purposes in order to better understand what affects forestomach motility.

Relationship between Passage Rate, Digestibility, and Motility

Motility of the reticulorumen functions to mix ingesta, sort particles, and move feedstuff matter to the omasum. When motility is decreased, mixing of contents would also decrease. This could result in a reduced rate of fermentation and thus, feedstuff breakdown. Because forage particles would need more time in the reticulorumen to reach an adequate size for passage into and through the omasum, retention time would likely increase resulting in a decline in intake. One could assume that decreased intake, mixing, and fermentation could reduce the ability of the animal to get nutrients resulting in poor average daily gain. On the other hand, an increased retention time may increase digestibility of the feedstuff providing more nutrients to the animal. Yet, the poor mixing of rumen contents may offset any positive effects on digestibility from increased retention time.

Water buffalo (*Bubalus bubalis*) had fewer A and B contraction sequences than cattle (*Bos indicus*; McSweeney et al., 1989). However, this was unable to reduce passage rate as mean retention time actually decreased 30% due to more rumination and greater quantities of fine particles in buffalo. Cold exposure increases feed intake, passage rate, and motility (Christopherson and Kennedy, 1983). Nonetheless, the actual role of motility in this response is unknown.

When conducting research regarding the effect of motility on passage rate, the duration of contractions should be measured. Okine et al. (1989) determined that frequency of reticular contractions of Hereford steers did not alter passage rate of ruminal fluid or particulate matter. However, duration of reticular contractions, a

parameter not typically measured in motility studies, appeared to be an important factor affecting passage rate.

Physiological Control

Regulation of reticuloruminal and gastrointestinal tract motility are extremely complicated and full coverage on this subject is beyond this review. Numerous locations and compounds for regulation likely exist since reticuloruminal motility is controlled by the extrinsic and intrinsic autonomic nervous systems (Grovum, 1986). Primary contraction form, frequency, and amplitude are controlled centrally (Leek and Harding, 1975). Yet, peripheral plexii are also important. Evidence for peripheral influences was provided by the reticuloruminal motility existence in chronically vagotomized sheep (Duncan, 1953; Ruckebusch et al., 1972; Gregory, 1982), induction of motility with distention of the rumen (Ruckebusch et al., 1972; Gregory, 1984a), elimination of motility with atropine (Gregory, 1984a), and inhibition of motility with high concentrations of luminal undissociated volatile fatty acids (Gregory, 1984b). Therefore, neuronal control of reticulorument motility is mediated through both central and peripheral neurotransmitters and receptors.

Due to their binding abilities with neurotransmitter receptors, ergot alkaloids have potential to influence reticuloruminal motility. Intravenous administration of ergopeptide alkaloids, ergovaline and ergotamine, reduced contractions of the sheep forestomach (McLeay and Smith, 2006; Poole et al., 2009). Further investigations are needed to explicitly deduce the relationships between motility, passage rate, and digestibility of endophyte-infected tall fescue to determine if or how dietary ergot alkaloids alter reticulorumen motility and subsequent ruminal fermentation.

Conclusion

The consumption of endophyte-infected tall fescue and related fescue toxicosis syndrome has sparked a vast amount of research since the mid-1900s due to its significant impact on the grazing industry, especially the beef cattle industry, in the United States. A fungal endophyte which infects tall fescue provides agronomic benefits to the grass. However, the endophyte produces ergot alkaloids that cause serious efficiency and performance losses in animal production systems. Many of the effects of ergot alkaloids can be attributed to structural similarities between ergot alkaloids and monoamine neurotransmitters. Agonist action on dopaminergic and serotonergic receptors enables ergot alkaloids to affect a variety of biological and physiological functions.

Copyright © Amanda M. Egert 2014

Chapter 3: Dietary exposure to ergot alkaloids decreases contractility of bovine mesenteric vasculature[1]

Introduction

Neotyphodium coenphialum is an endophyte symbiotically associated with tall fescue grass (*Lolium arundinaceum*) (Porter et al., 1979; Bush et al., 1982). The endophyte, although conveying beneficial properties to the grass, such as insect and drought resistance, produces a number of ergot alkaloids which cause vasoconstriction and fescue toxicosis in grazing animals (Lyons et al., 1986; Strickland et al., 2011). Ergot alkaloids induce vasoconstriction in bovine core (Rhodes et al., 1991), peripheral (Solomons et al., 1989; Klotz et al., 2006; Aiken et al., 2007), and foregut (Foote et al., 2011) blood vessels and have been shown to decrease blood flow to the rumen epithelium (Foote et al., 2013). Westendorf et al. (1993) reported that 50 to 60% of ergot alkaloids administered in the diet are recovered in the abomasum; thus, it follows that ergot alkaloids enter the small intestine. However, little information is available about the effect of these compounds on the small intestine or what interaction alkaloids might have with the midgut. Klotz et al. (2012) demonstrated a reduction in contractile response to 5- hydroxytryptamine (**5HT**; serotonin) and ergovaline in lateral saphenous veins collected from steers that had grazed high-endophyte tall fescue. Therefore, it was hypothesized that ergot alkaloids cause vasoconstriction in midgut vasculature and that prior dietary exposure to ergot alkaloids may decrease their vasoactivity. The objectives of this study were to profile the vasoactivity of ergot alkaloids in mesenteric artery (**MA**)

[1] Published with permission from the Journal of Animal Science
Egert, A. M., D. H. Kim, F. N. Schrick, D. L. Harmon, and J. L. Klotz. 2014a. Dietary exposure to ergot alkaloids decreases contractility of bovine mesenteric vasculature. J. Anim. Sci. 92:1768-1799.

and vein (**MV**) and to determine if prior dietary exposure to toxic endophyte-infected tall fescue seed affected in vitro vasoactivity of ergocryptine (**ERP**), ergotamine (**ERT**), ergocristine (**ERS**), ergocornine (**ERO**), ergonovine (**ERN**), lysergic acid (**LSA**), an ergovaline-containing tall fescue seed extract (**EXT**), and 5HT.

Materials and Methods

The procedures used in this study involving live animals were approved by the University of Kentucky Institutional Animal Care and Use Committee.

Animals and Seed Treatment

Twelve ruminally cannulated Angus steers (BW = 547 ± 31 kg) were paired by weight into 6 blocks and housed at the University of Kentucky C. Oran Little Research Center in Woodford Co. KY. Steers were fed a basal diet of alfalfa cubes (% DM basis: CP = 16.5; ADF = 37.2; NDF = 51.9; NE_m = 5.19 MJ/kg) at 1.5 × NE_m once daily at 0700 h; the diet was topdressed with a mineral pre-mix (Kentucky Nutrition Service, Lawrenceburg, KY, USA; NaCl = 92%; Zn = 5500 mg/kg; Fe = 9275 mg/kg; Mn = 4790 mg/kg; Cu = 1835 mg/kg; I = 115 mg/kg; Se = 18 mg/kg; Co = 65 mg/kg). Steers were given free access to water. Within each block, 1 steer was randomly assigned to an endophyte-infected (**E+**; **'**KY 31') tall fescue seed treatment, and the other was assigned to an endophyte-free (**E-**; 'KY 32') tall fescue seed treatment. Tall fescue seed was ground by a grinder mixer (MX125, Gehl, West Bend, WI) to pass through a 1.25-cm screen. Steers were dosed through the rumen cannula immediately after feeding with 1 kg of E- (0 mg ergovaline + ergovalinine/kg DM) or E+ (4.45 mg ergovaline + ergovalinine/kg DM) seed daily for 21 d before slaughter. The starting points of feeding

periods were staggered to accommodate slaughter of 1 block per week with 1 d in between slaughters within a week to allow for completion of myograph experiments.

Blood Collection and Analysis

Blood samples were collected from steers immediately before feeding on d 14 via a catheter in the jugular vein. The blood was permitted to clot for 24 h at 4°C and centrifuged (1,500 × *g*) for 25 min at 4°C. Analysis of serum prolactin concentrations was conducted by the RIA procedures of Bernard et al. (1993). Intra- and interassay CV were 6.6% and 7.1%, respectively.

Tissue Collection and Preparation

On d 22, steers were slaughtered at the University of Kentucky Meat Laboratory (E+: BW = 564 ± 16 kg; E-: BW = 585 ± 8 kg). Shortly after the gastrointestinal tract was removed from the carcass, branches of MA and MV supporting the cranial portion of the ileum were collected, placed in a modified Krebs-Henseleit buffer (95% O_2/5% CO_2; pH = 7.4; 11.1 m*M* *D*-glucose; 1.2 m*M* $MgSO_4$; 1.2 m*M* KH_2PO_4; 4.7 m*M* KCl; 118.1 m*M* NaCl; 3.4 m*M* $CaCl_2$; 24.9 m*M* $NaHCO_3$; Sigma Chemical Co., St. Louis, MO) on ice, and transported to the laboratory. Mesenteric artery and MV were separated and carefully cleaned of fat and connective tissue. Samples were sectioned into approximately 2-mm segments and examined under 12.5× magnification using a dissecting microscope (Stemi 2000-C, Carl Zeiss Inc., Oberkochen, Germany). At that time, segments of vessel that were found to have abnormalities or to be structurally compromised were replaced with an uncompromised segment. Due to the noncircular

nature of MV, vascular measurements were recorded for MA cross-sections only using Axiovision (version 20, Carl Zeiss Inc.).

Standards and Concentrations

Stock solutions of ERP, ERT, ERS, ERO, ERN, EXT, LSA, and 5HT were diluted to corresponding concentrations for final working concentrations in the tissue bath of 5×10^{-10} to 1×10^{-6} *M* for EXT and 5×10^{-9} to 1×10^{-4} *M* for all other agonists in the tissue bath. Alpha-ergocryptine (99%; E5625; Sigma Chemical Co.), ERT (ergotamine D-tartrate; ≥97%; 45510; Aldrich, Milwaukee, WI), ERS (ergocristine; Research Plus, Barnegat, NJ), ERO (ergocornine; 95%; E131; Sigma Chemical Co.), and ERN (ergonovine maleate salt; 100%; E6500; Sigma Chemical Co.) were prepared in dimethylsulfoxide. The EXT and LSA (d-lysergic acid; 95%; Acros Organics, Geel, Belgium) were prepared in 80% methanol, and 5HT (serotonin hydrochloride; 100%; H9523; Sigma Chemical Co.) was prepared in H_2O. Because of limited availability of purified ergovaline, an ergovaline-containing tall fescue seed extract was used. Foote et al. (2012) described the ultra-performance liquid chromatography/tandem mass spectrometry (Acquity UPLC-TQD; Waters, Inc., Milford, MA) quantitative analysis of the alkaloid concentrations of this extract (the same extract lot was used in the current study) and also reported that the contractile response of bovine lateral saphenous vein to an E+ seed extract and ergovaline alone were not different. Because the starting concentration of ergovaline in EXT was fixed (the maximum starting concentration was 1×10^{-6} *M*), only 8 standard additions were used for EXT. For all other agonists, there were a total of 10 standard additions. All additions were added to myograph chambers in order of increasing concentration.

Experimental Myograph Protocol

Vessels were mounted onto luminal supports in individual chambers of a multimyograph (DMT 610M, Danish Myo Technology, Atlanta, GA) with 5 mL Krebs-Henseleit buffer and constant gassing (95% O_2/5% CO_2; pH = 7.4; 37°C). The incubation buffer included the transport buffer composition as well as 3×10^{-5} *M* desipramine (D3900; Sigma Chemical Co.) to inactivate reuptake of catecholamines by neurons and 1×10^{-6} *M* propranolol (P0844; Sigma Chemical Co.) to block β-adrenergic receptors. An equilibration period of 90 min under the above conditions with buffer changes every 15 min was given to allow the vessels to equilibrate at a resting tension of approximately 1 g. Experimental tissues were exposed to a reference addition of 120 m*M* KCl (Sigma Chemical Co.) for 15 min to confirm tissue viability and responsiveness. Tissues were washed with incubation buffer every 15 min until the vessels returned to approximately 1 g tension. Once the return to resting tension had been reached, the standard additions were started for cumulative contractile response experiments. Treatments were added in 15-min cycles that consisted of a 9-min treatment incubation period, followed by two 2.5-min buffer washes, a final buffer replacement, and a 1-min recovery before the next standard addition. At the completion of the experiment, the vessels were once again exposed to 120 m*M* KCl to verify that vessels were viable for the duration of the experiment.

Data Collection and Analysis

Isometric contractile response of mesenteric vessels to KCl, ERP, ERT, ERS, ERO, EXT, ERN, LSA, and 5HT were digitized and recorded in grams of tension using

Powerlab/8sp and Chart software (version 7.3, ADInstruments, Colorado Springs, CO). For each standard addition, the maximum tension (g) during the 9-min incubation period was recorded as the contractile response, which was then corrected for baseline tension as established immediately before the 120 m*M* KCl reference addition. Because of variations between animals and in tissue size, the contractile response was normalized as a percentage of the maximum grams of tension induced by the KCl reference addition to compensate for differences in tissue responsiveness. Response data are presented as the percentage mean contractile response $\pm$ SEM of the maximum contractile response produced by the 120 m*M* KCl reference addition. Sigmoidal concentration response curves of MA and MV to each ergot alkaloid were calculated and plotted using non-linear regression with variable slope (GraphPad Prism 5, GraphPad Software Inc., La Jolla, CA). This tabulation involved a 3-parameter equation:

$$y = bottom + \frac{(top - bottom)}{\left[1 + 10^{(\log EC_{50} - x)}\right]},$$

where top and bottom are the percentage of 120 m*M* KCl maximum contractile response at the plateaus, and the EC_{50} is the molar concentration of agonist producing 50% of the KCl maximum response. Thus, the EC_{50} is a measure of potency of a compound. For ease of understanding and to correspond with the response curve graphs, results were presented as the negative log of the EC_{50} value.

Statistical Analysis

All statistical analyses were done using the MIXED model of SAS (SAS 9.3, SAS Inst. Inc., Cary, NC). Serum prolactin concentrations for E+ and E- treated steers were compared using a randomized block design. The maximum contractile response of MA

and MV to 120 m*M* KCl, as well as the inner and outer diameters of MA (analyzed separately), were analyzed using a randomized block with replication design for the effect of seed. Contractile response to agonist treatments for MA and MV were analyzed separately as a randomized block with a split-plot treatment design. The whole plot experimental unit was steer with seed as the treatment factor. Vessel was the subplot experimental unit, and alkaloid concentration was the subplot treatment factor. Model terms included the fixed effects of seed treatment, agonist concentration, and the interaction of seed × concentration. Analysis of the negative log EC_{50} for each agonist was conducted using a randomized block with a split-plot treatment arrangement, and terms of the model included seed, agonist, and the interaction.

For all analyses, pairwise comparisons of least square means (± SEM) were conducted if the probability of a greater *F*-statistic in the ANOVA was significant for the tested effect. The LSD feature of SAS was performed for mean separation. Results denoted as being significant have probabilities $P < 0.05$, unless otherwise reported.

Results

Steers treated with E+ seed had a lower ($P = 0.05$) mean serum prolactin concentration than the E- steers (E+: 0.48 ± 14.36 ng/mL; E-: 50.16 ± 14.36 ng/mL). Mean i.d. of MA was less ($P = 0.04$) for E+ steers than for E- steers (E+ = 0.62 ± 0.04 mm; E- = 0.74 ± 0.04 mm). Similarly, the mean o.d. of MA was smaller ($P = 0.02$) for E+ steers than E- steers (E+: 1.54 ± 0.04 mm; E-: 1.69 ± 0.04 mm). The maximum contractile response to 120 m*M* KCl was not different between seed treatments for MA ($P = 0.33$; E-: 2.67 ± 0.43 g; E+: 3.33 ± 0.43 g) or MV ($P = 0.26$; E-: 2.01 ± 0.18 g; E+: 1.81 ± 0.18 g).

All *P*-values reported for agonist treatment contractile responses are for the seed × concentration interaction, unless otherwise specified. All agonists were vasoactive in MA and MV of E- steers with the exception of LSA. In contrast, vessels of E+ steers did not respond to several agonists. Specifically, contractile responses to ERP were less ($P < 0.01$) in MA (Figure 3.1A) and MV (Figure 3.1B) from E+ steers than E- steers. Similarly, E+ steers had a significantly reduced contractile response to ERT in MA ($P < 0.01$; Figure 3.2A) and MV ($P = 0.03$; Figure 3.2B). Response to ERS was also decreased for E+ steers compared to E- steers in MA ($P < 0.01$; Figure 3.3A) and MV ($P = 0.05$; Figure 3.3B). The E+ steers had a reduced contractile response to ERO in MA ($P < 0.01$; Figure 3.4A), but this was not seen in MV ($P = 0.998$; Figure 3.4B).

The MA and MV of E+ steers had decreased ($P < 0.01$) contractile responses to ERN (Figure 3.5A and 3.5B). There was a tendency for E- to have a greater contractile response in MA when exposed to LSA (P = 0.105; Figure 3.6A). In MV, LSA produced a significant concentration effect ($P < 0.01$), but this was due to a reduction in tension over the period of the experiment and not a contractile response (Figure 3.6B).

The EXT, based on concentration of ergovaline, induced a larger isometric contraction in MA ($P < 0.01$; Figure 3.7A) and MV ($P = 0.04$; Figure 3.7B) of E- steers than E+ steers. Likewise, contractile response in MA and MV of E+ steers was less ($P < 0.01$) than that of E- steers when exposed to 5HT (Figure 3.8A and 3.8B). 5-Hydroxytryptamine (1×10^{-4} *M* addition) produced a very large contractile response, with MA of E- steers reaching approximately 70% and MV of E- steers reaching approximately 90% of the maximum contractile response induced by KCl.

For EC_{50} analysis, an effect of agonist was present in MA ($P < 0.01$), without a seed ($P = 0.95$) or seed × agonist effect ($P = 0.90$; Table 3.1). The EC_{50} value for ERN

was not different from that of ERS or ERO in MA. Similarly, MV also showed an agonist effect (P <0.01). Seed treatment did not affect potency in MV (P = 0.80), and there was a tendency (P = 0.10) for an interaction. The response of MA to 5HT was different from all other agonist treatments evidenced by a lower –log EC_{50} value. This indicates 5HT reached 50% of the maximum contractile response at a greater concentration than other agonists and thus, arteries were less sensitive to 5HT than the other agonists tested. However, MV did not show a similar pattern as the EC_{50} for 5HT was only different from ERO and EXT. As expected from previous myograph experiments with bovine vasculature, the artery and vein varied in sensitivity to the agonist treatments.

The EXT was the most potent agonist in MV of E- and E+ steers with –log EC_{50} values of 7.26 ± 0.42 and 7.09 ± 0.42 *M*, respectively (Table 3.1). Negative log EC_{50} values of ergopeptines, ERP, ERT, ERS, and ERO, were not significantly different from one another in MV. However, another ergopeptine, the ergovaline in EXT, had a greater (P < 0.05) –log EC_{50} compared to the 4 pure ergopeptines previously mentioned, signifying MV of steers were more sensitive to EXT than the other ergopeptine agonists tested (Table 3.1).

Discussion

This experiment was the first to demonstrate that ergot alkaloids were vasoactive in mesenteric vasculature in vitro using a bovine model. To distinguish differences between E+ and E- steers, it was important that the experimental model showed the E+ seed treatment provided an adequate quantity of ergot alkaloids to induce fescue toxicosis. Research has shown that most animals consuming endophyte-infected tall fescue or ergot alkaloids have decreased serum prolactin concentrations (Hurley et al.,

1980). Normally, dopamine inhibits prolactin secretion by interacting with D2 dopamine receptors (Lamberts and Macleod, 1990). However, structural similarities of the ergoline ring structure of ergot alkaloids compared to dopamine allow ergot alkaloids to bind and act upon D2 dopamine receptors (Berde and Stürmer 1978; Goldstein et al., 1980; Sibley and Creese, 1983) in the anterior pituitary gland and to inhibit secretion of prolactin (Schillo et al., 1988; Porter and Thompson, 1992). Thus, decreased serum prolactin has frequently been used as a broad indicator of fescue toxicosis in cattle, but the degree of decrease does not indicate level of ergot alkaloid exposure. The reduced blood prolactin concentrations (on d 14) of E+ steers in this study suggest that E+ seed dosed steers were experiencing effects of fescue toxicosis at the time of vessel harvest. Additionally, evidence of vasoconstriction of mesenteric vasculature due to E+ seed treatment was demonstrated by the mesenteric artery of E+ steers having smaller i.d and o.d. than the E- steers. Similarly, Klotz et al. (2012) showed a decrease in i.d. of the bovine lateral saphenous vein when steers grazed a high-endophyte tall fescue pasture compared to a low-endophyte mixed-grass pasture; however, no effect of endophyte level on o.d was present. Several researchers also found that ergot alkaloid exposure produced morphological changes in vasculature of livestock and thickening of small peripheral blood vessels (Julien et al., 1974; Williams et al., 1975; Garner and Cornell, 1978). On the basis of the results presented here and reported in the literature, the seed treatment was effective at inducing fescue toxicosis. The decreased size of mesenteric arteries in ergot alkaloid-exposed cattle in this study could have possibly led to a decline in blood flow. In support of this hypothesis, Rhodes et al. (1991) showed that steers had reduced blood flow to the duodenum and other tissues (cerebellum, skin over the ribs, and colon) after consuming a high-endophyte diet. Likewise, sheep on a 30-d treatment of high-endophyte diet also displayed reduced blood flow to the adrenal glands and skin of the inner hind leg (Rhodes et al., 1991). The gastrointestinal tract relies on splanchnic

blood flow for its functions (Matheson et al., 2000). As digesta move across the mucosal surface, arterial blood flow to that area increases to aid in nutrient absorption (Chou et al., 1992). Thus, it is conceivable that the vasoconstriction evident in the E+ steers could have impaired nutrient uptake and transport by decreasing blood flow to and from mesenteric tissues.

Ergopeptines, including ERP, ERT, ERS, and ERO, if they induced a contractile response in mesenteric vasculature, appeared to begin demonstrating a response around 1×10^{-6} *M*, and the maximum contractile intensity was around 20 to 40% of the KCl maximum. Ergonovine, an ergoline alkaloid, appeared to have an onset of contraction at a greater concentration (5×10^{-6} *M*) than that of the ergopeptines yet also reached between 20 to 40% of the KCl maximum response. Therefore, ERN generally had lower –log EC_{50} values than ergopeptine alkaloids (with the exception of ERS) in MV of E- steers signifying it reached 50% of its maximum contractile response at a greater concentration and is therefore less potent. In contrast, research done using lateral saphenous veins from fescue-naïve cattle showed ERN induced the greatest maximal contractile response of the agonists tested, and the EC_{50} value for ERN was not different from ERP or ERS (Klotz et al., 2010). Likewise, ERN displayed similar EC_{50} values to ERP, ERO, and ERS in bovine ruminal artery (Foote et al., 2011). Also, MA of steers displayed similarities in contractile response and potency between ERN and ergopeptine alkaloids, particularly ERS and ERO. Therefore, it appears ERN behaves similarly to ergopeptine alkaloids. Differences in results between ERN and ergopeptine alkaloids could possibly be attributed to ERN lacking the peptide moiety of ergopeptine alkaloids which can affect binding mode and affinity (Choudhary et al., 1995). An example of this difference between ergoline and ergopeptine alkaloids can be demonstrated by bovine vesicular glutamate transporters (**VGLUT**) activity, which collects L-glutamate into

synaptic vesicles for stimulation of glutamatergic neurons. The VGLUT activity was decreased 80 to 90% by ergovaline, ERO, ERT, and bromocriptine (synthetic ergopeptine alkaloid; Xue et al., 2011). Conversely, a separate study showed alkaloids without the peptide moiety were poor inhibitors of VGLUT activity (Carlson et al., 1989).

Although ERN and LSA are structurally similar ergoline alkaloids, ERN produced a contractile response, and LSA failed to induce vasoconstriction. This lack of vasoactive response after exposure of mesenteric vasculature to LSA agrees with other studies which showed LSA did not induce significant vasoconstriction in bovine right ruminal artery and vein (Foote et al., 2011), and LSA produced only a slight contractile response in bovine lateral saphenous vein at supraphysiological concentrations (Klotz et al, 2006). On the basis of this compilation of evidence, the hypothesis that LSA has no direct vasoconstrictive effect on bovine vasculature was supported.

Ergovaline is among the most researched ergot alkaloids in relation to fescue toxicosis and vasoconstriction, as it is one of the most toxic alkaloids and makes up approximately 57 to 73% of the total ergopeptines in endophyte-infected tall fescue (Yates et al., 1985; Bacon et al., 1986). Based on results of Foote et al. (2012), the contractile response of mesenteric vasculature generated with EXT could be attributed to ergovaline alone, not the combination of alkaloids. In this experiment, EXT reached approximately the same maximum contractile response as the other ergopeptines. However, this maximum was achieved at a much lower concentration than the other ergopeptines required to reached their maximum at (1×10^{-6} versus 1×10^{-4} *M*, respectively), demonstrating that the ergovaline in EXT is a more potent vasoconstrictor in bovine mesenteric vasculature than the other ergopeptines tested. Further evidence for this increased potency of ergovaline was provided by the EXT having the greatest – log EC_{50} values for all seed treatment and vessel types for which data were available.

Ergovaline was previously found to be 75 times more potent than 5HT at inducing vasoconstriction of the uterine artery from pregnant cows (Dyer, 1993). Previous myograph studies have similarly shown evidence of ergovaline as a very potent vasoconstrictor of bovine vasculature (Klotz et al., 2007, 2008; Foote et al., 2011; Foote et al., 2012).

A consistent observation across both MA and MV in the current study was a reduced or abolished contractile response in the vessels from E+ treated steers. This is the first report of this in mesenteric vasculature, but in 2 separate grazing trials, Klotz et al. (2012; 2013) reported similar observations for reduced contractile responses of lateral saphenous veins to ergovaline and 5HT in E+ steers compared to E- steers. This reduced contractile response could be a result of changes in involved receptor populations and activity, physical limitations due to vasoconstriction, or changes in gene expression related to secondary messaging systems associated with G protein-coupled receptors (GPCR). Dyer (1993) proposed that activation of 5HT receptors could be the main cause for the toxic effects of endophyte-infected fescue after finding ketanserin, a $5HT_{2A}$ antagonist, reduced the contractile response of bovine uterine and fetal umbilical arteries to ergovaline, whereas specific α-adrenergic antagonists did not. Likewise, Schöning et al (2001) showed ERT and ergovaline interact with $5HT_{2A}$, $5HT_{1B/1D}$, and α_1-adrenergic receptors to cause vasoconstriction of a rat tail artery. Our study provides strong evidence that prior alkaloid exposure has an effect on 5HT vasoactivity because endophyte-infected seed dosed steers had a considerably decreased contractile response to 5HT in mesenteric vasculature compared to endophyte-free dosed steers. Klotz et al. (2012) demonstrated a similar occurrence in peripheral vasculature; bovine lateral saphenous veins of steers grazed on a high-endophyte tall fescue pasture exhibited reduced contractile responses to 5HT and ergovaline compared to those on

low-endophyte pasture. Based on evaluation of different serotonin receptor subtype agonists, Klotz et al. (2012) indicated that the $5HT_{2A}$ and $5HT_7$ subtypes are involved in vasoconstriction of fescue toxicosis and are affected by chronic ergot alkaloid exposure via grazing. Because the mesenteric arteries of endophyte-infected dosed steers were smaller than endophyte-free dosed steers and thus vasoconstriction was already evident before myograph treatments, 1 hypothesis for the observed reduction in contractile response of E+ steers to agonist treatments was that the blood vessels of E+ steers were physically unable to contract as much as the E- treated vessels. However, comparison of the KCl maximum data for mesenteric artery and vein provides evidence that this was not true, as there was no effect of seed treatments on the maximum contractile responses to KCl for mesenteric artery or vein.

Although still not fully understood, many studies have focused on determining the mechanism by which ergot alkaloids induce vasoconstriction of blood vessels. It is known that ergot alkaloids interact with biogenic amine receptors (Weber, 1980; Pertz and Eich, 1999), such as dopaminergic (Larson et al., 1995), serotonergic (Dyer, 1993), α_1-adrenergic (Schöning et al., 2001), and α_2-adrenergic (Oliver et al., 1998) receptors, to induce vascular effects and alter cellular activity. Receptors such as these are G protein-coupled transmembrane proteins with 7 helical domains (Goddard and Abrol, 2007) which elicit secondary messenger responses upon ligand binding. A report demonstrated that ergot alkaloids (ergovaline, ERP, ERT, and ERN), binding to D_2dopamine receptors inhibited the vasoactive intestinal peptide-stimulated production of cyclic adenosine monophosphate (**AMP**) in GH_4ZR_7 cells (Larson et al., 1995), which would reduce the intracellular concentration of cyclic AMP, thereby affecting signal transduction pathways and ultimately, cellular function. Furthermore, numerous studies have shown that alkaloids can bind receptors in what appears to be an irreversible

association with very slow dissociation from receptor sites (Silberstein, 1997; Schöning et al., 2001; Klotz et al., 2007). Ergovaline bioaccumulation within bovine peripheral vasculature after repetitive exposure has been demonstrated in vitro, but the same phenomenon did not occur with LSA (Klotz et al., 2009). Additionally, Mulac and Humpf (2011) found that accumulation of ergopeptine alkaloids occurred in relation to necrotic and apoptotic effects in human primary cells. Based on this information, it is possible that ergot alkaloids from the dietary exposure in our study may have remained bound to the receptors, eventually leading to a reduction in receptors on the cell surface due to an imbalance between receptor internalization and trafficking of new receptors to the cell surface (Tan et al., 2004). As a result, this could have reduced the number of available receptors in the vessel tissue for further alkaloid binding and subsequent vasoconstriction, thereby decreasing the observed contractile response to myograph treatments. Another plausible explanation for this occurrence may be a down regulation of gene transcription for GPCR elicited by ergot alkaloids at the mRNA level (Maurer, 1981). Additionally, ergot alkaloids appear to have a variety of mechanisms of action on receptors and second messengers with both agonistic and antagonistic effects in target tissues (Pertz and Eich, 1999; Schöning et al., 2001) which have made finding 1 solution to the fescue toxicosis problem difficult.

Conclusions

This study demonstrated that ergot alkaloids, with the exception of lysergic acid, were vasoactive in the bovine midgut mesentery in vitro. Steers exposed to endophyte-infected tall fescue seed had diminished or lacked contractility to many ergot alkaloids and 5HT in the small intestinal vasculature. This suggests that dietary exposure to ergot alkaloids could affect absorption of nutrients within the animal by decreased blood flow

to and from the midgut due to vasoconstriction. However, because steers which had already been dosed with endophyte-infected tall fescue seed exhibited a reduced contractile response in vitro compared to endophyte-free dosed steers, the vasoactive response to ergot alkaloids and biogenic amines is suppressed in chronically exposed cattle. Prevention of midgut absorption of ergot alkaloids is viewed as a viable solution to the fescue toxicosis syndrome. Along with determining the mechanism of ergot alkaloid absorption from the midgut, further research should be directed towards investigating the passage of nutrients through mesenteric vasculature of animals exposed to endophyte-infected and endophyte-free tall fescue seed.

Table 3.1 The -log EC_{50} (M) means and SEM for agonists in mesenteric artery and vein.[1,2,3]

Myograph Treatment	-Log EC_{50}, *M*						Effect		
	E-[4]			**E+**[4]			Seed	Agonist	Seed × Agonist
Artery							0.95	<0.01	0.90
Ergocryptine	5.23	± 0.40	(6)	NR			-	-	-
Ergotamine	6.03	± 0.40	(6)	5.77	± 0.40	(6)	-	[c]	-
Ergocristine	5.23	± 0.40	(6)	5.61	± 0.48	(4)	-	[bc]	-
Ergocornine	5.74	± 0.40	(6)	5.57	± 0.43	(5)	-	[bc]	-
Ergonovine	5.08	± 0.40	(6)	4.79	± 0.48	(4)	-	[b]	-
Extract[5]	ND			7.14	± 0.55	(3)	-	-	-
5HT[6]	3.14	± 0.68	(2)	3.39	± 0.43	(5)	-	[a]	-
Vein							0.80	<0.01	0.10
Ergocryptine	5.94	± 0.34	(5)	5.95	± 0.43	(3)	-	[ab]	-
Ergotamine	6.48	± 0.38	(4)	5.40	± 0.38	(4)	-	[ab]	-
Ergocristine	5.74	± 0.31	(6)	5.62	± 0.34	(5)	-	[ab]	-
Ergocornine	6.27	± 0.34	(5)	6.05	± 0.34	(5)	-	[b]	-
Ergonovine	5.10	± 0.34	(5)	NR			-	-	-
Extract	7.26	± 0.43	(3)	7.09	± 0.43	(3)	-	[c]	-
5HT[6]	4.60	± 0.34	(5)	5.87	± 0.43	(3)	-	[a]	-

[abc] Within a column for artery, means without a common superscript differ ($P < 0.05$).
[abcd] Within a column for vein, means without a common superscript differ ($P < 0.05$).

[1] -Log EC_{50} = measure of potency of an agonist, expressed as the molar concentration of that agonist required to produce 50% of the maximum contractile response produced by the reference compound, in this case 120 m*M* KCl.
[2] Lysergic acid failed to induce vasoconstrictive response, so it was not included in the data set for analysis of -log EC_{50} values.
[3] Some data were unable to be fit to a sigmoidal curve response preventing the calculation of –log EC_{50} values due to no response (NR). Therefore, variable numbers of experimental replicates resulted, which are denoted in parenthetical values after the SEM for –log EC_{50} values.
[4] Seed treatment: Angus steers (n = 12) were paired by weight into six blocks. Within each block, one steer was dosed ruminally with 1 kg endophyte-free (**E-**) tall fescue seed and the other with the same quantity of endophyte-infected (**E+**) tall fescue seed.
[5] Due to ambiguity of results from sigmoidal regression, EC_{50} values were not determined (ND) for extract treatment of endophyte-free (E-) seed treated steers.
[6] 5HT = 5-Hydroxytryptamine

Figure 3.1. Mean contractile response of mesenteric artery (A) and vein (B) to increasing concentrations of ergocryptine (**ERP**) for endophyte-infected (**E+**) and endophyte-free (**E-**) tall fescue seed-treated steers. A) For artery, effects of seed, concentration, and seed × concentration were significant ($P < 0.04$). B) For vein, effects of seed, concentration, and seed × concentration were significant ($P < 0.01$). Regression lines shown are due to using non-linear regression analysis to fit the data to a sigmoidal concentration response curve which utilized the following equation: $y = \text{bottom} + [(\text{top} - \text{bottom}) / (1 + 10^{(\log EC_{50} - x)})]$, where top and bottom are the percentage of 120 mM KCl maximum contractile response at the plateaus, and the EC_{50} is the molar concentration of alkaloid producing 50% of the KCl maximum response.

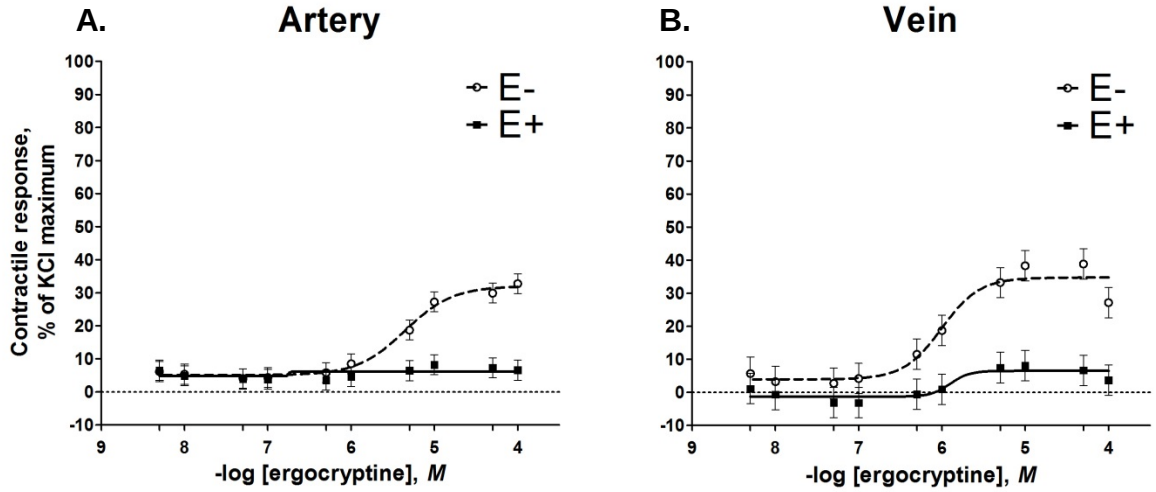


Figure 3.2. Mean contractile response of mesenteric artery (A) and vein (B) to increasing concentrations of ergotamine (**ERT**) for endophyte-infected (**E+**) and endophyte-free (**E-**) tall fescue seed-treated steers. A) For artery, effects of seed, concentration, and seed × concentration were significant ($P < 0.01$). B) For vein, effects of concentration and seed × concentration were significant ($P < 0.03$). The effect of seed was not significant ($P = 0.23$). Regression lines shown are due to using non-linear regression analysis to fit the data to a sigmoidal concentration response curve which utilized the following equation: $y = \text{bottom} + [(\text{top} - \text{bottom}) / (1 + 10^{(\log EC_{50} - x)})]$, where top and bottom are the percentage of 120 mM KCl maximum contractile response at the plateaus, and the EC_{50} is the molar concentration of alkaloid producing 50% of the KCl maximum response.

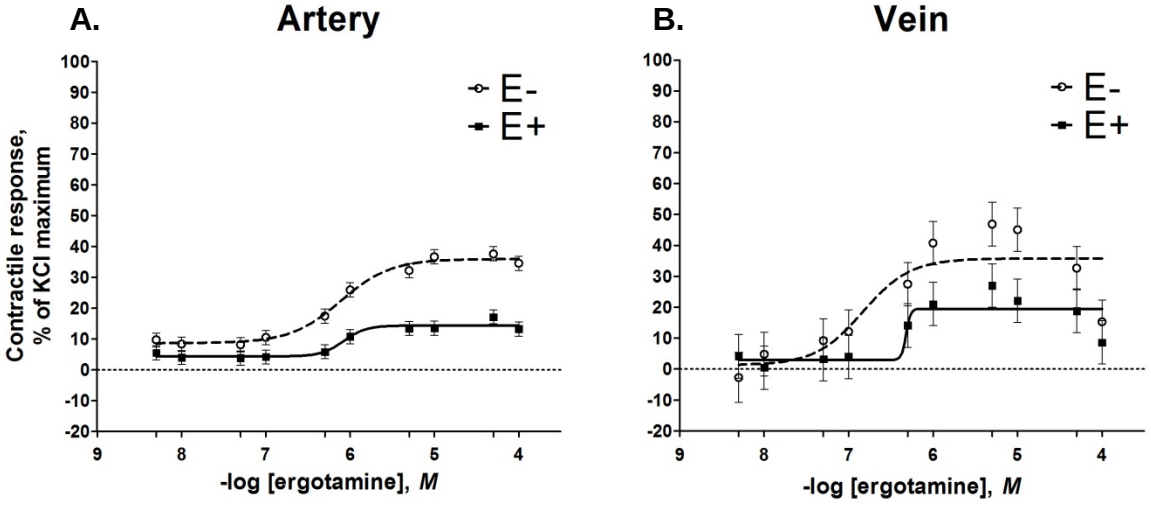


Figure 3.3. Mean contractile response of mesenteric artery (A) and vein (B) to increasing concentrations of ergocristine (**ERS**) for endophyte-infected (**E+**) and endophyte-free (**E-**) tall fescue seed-treated steers. A) For artery, effects of seed, concentration, and seed × concentration were significant ($P < 0.03$). B) For vein, effects of seed ($P = 0.01$), concentration ($P < 0.01$) and seed × concentration ($P = 0.05$) were significant. Regression lines shown are due to using non-linear regression analysis to fit the data to a sigmoidal concentration response curve which utilized the following equation: $y = \text{bottom} + [(\text{top} - \text{bottom}) / (1 + 10^{(\log EC_{50} - x)})]$, where top and bottom are the percentage of 120 mM KCl maximum contractile response at the plateaus, and the EC_{50} is the molar concentration of alkaloid producing 50% of the KCl maximum response.

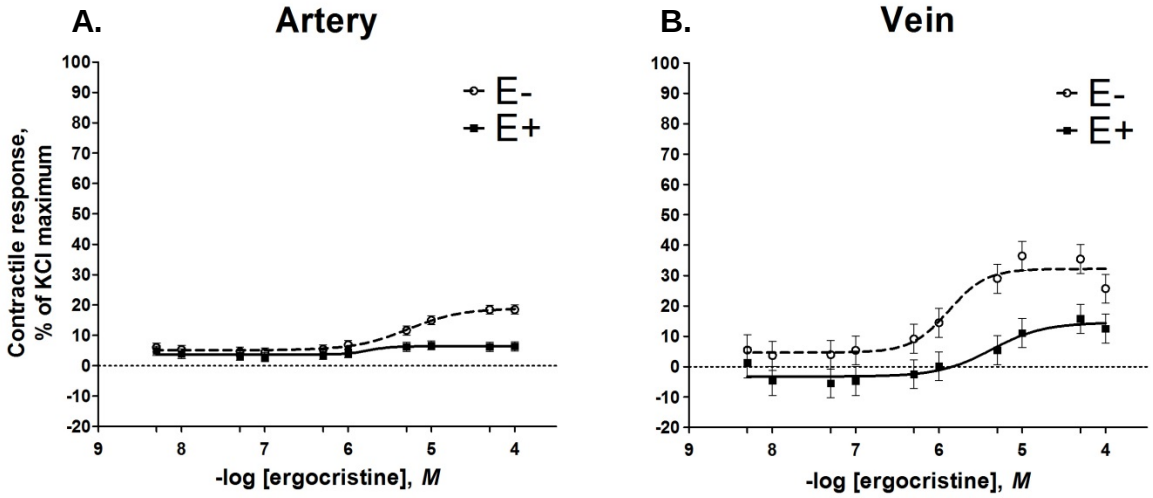


Figure 3.4. Mean contractile response of mesenteric artery (A) and vein (B) to increasing concentrations of ergocornine (**ERO**) for endophyte-infected (**E+**) and endophyte-free (**E-**) tall fescue seed-treated steers. A) For artery, effects of seed, concentration, and seed × concentration were significant ($P < 0.01$). B) For vein, the effect of concentration was significant ($P < 0.01$); however, seed ($P = 0.37$) and seed × concentration ($P = 0.998$) were not. Regression lines shown are due to using non-linear regression analysis to fit the data to a sigmoidal concentration response curve which utilized the following equation: $y = \text{bottom} + [(\text{top} - \text{bottom}) / (1 + 10^{(\log EC_{50} - x)})]$, where top and bottom are the percentage of 120 mM KCl maximum contractile response at the plateaus, and the EC50 is the molar concentration of alkaloid producing 50% of the KCl maximum response.

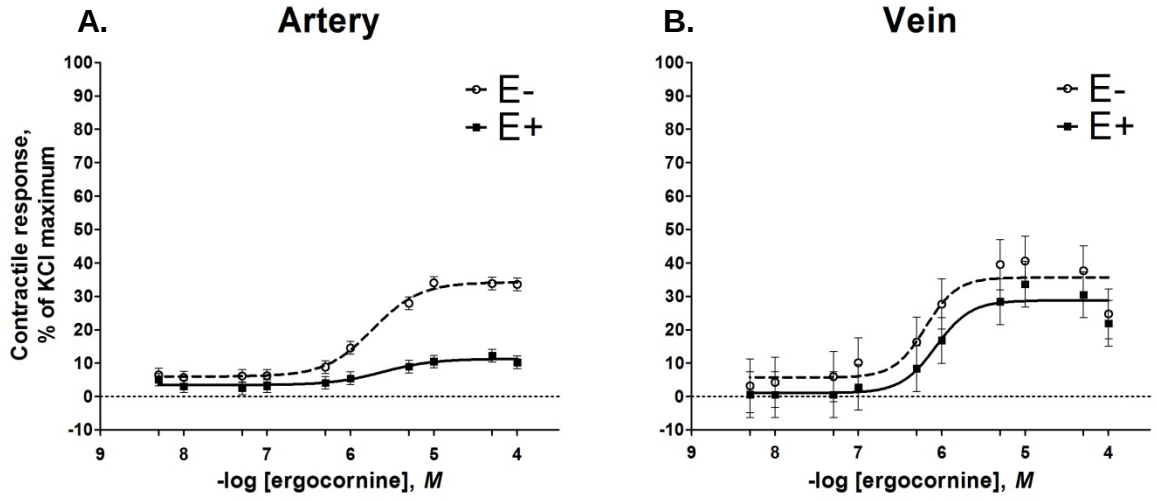


Figure 3.5. Mean contractile response of mesenteric artery (A) and vein (B) to increasing concentrations of ergonovine (**ERN**) for endophyte-infected (**E+**) and endophyte-free (**E-**) tall fescue seed-treated steers. A and B) Effects of seed, concentration, and seed × concentration were significant ($P < 0.01$). Regression lines shown are due to using non-linear regression analysis to fit the data to a sigmoidal concentration response curve which utilized the following equation: $y = bottom + [(top – bottom) / (1 + 10^{(logEC_{50} - x)})]$, where top and bottom are the percentage of 120 mM KCl maximum contractile response at the plateaus, and the EC_{50} is the molar concentration of alkaloid producing 50% of the KCl maximum response. Data without regression lines signifies the data could not be fit to the sigmoidal concentration response model.

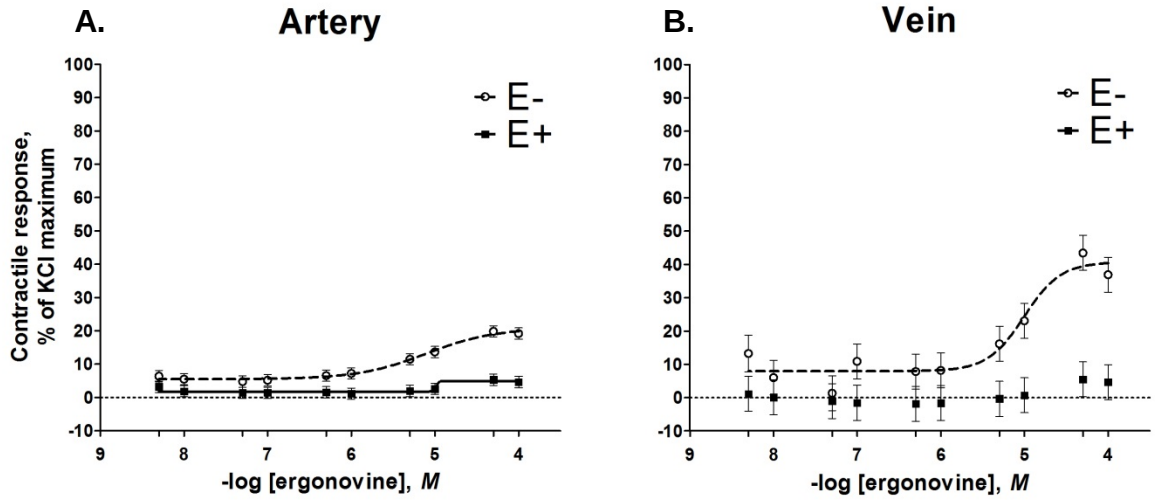


Figure 3.6. Mean contractile response of mesenteric artery (A) and vein (B) to increasing concentrations of lysergic acid (**LSA**) for endophyte-infected (**E+**) and endophyte-free (**E-**) tall fescue seed-treated steers. A) For artery, the effect of seed and concentration were significant ($P < 0.05$), and the interaction showed a tendency ($P = 0.105$), which was likely due to our experimental protocol and is not biologically significant. B) For vein, the effect of concentration was significant ($P < 0.01$); however, seed demonstrated a tendency ($P = 0.07$) and seed × concentration was not significant ($P = 0.13$). Regression lines shown are due to using non-linear regression analysis to fit the data to a sigmoidal concentration response curve which utilized the following equation: $y = \text{bottom} + [(\text{top} - \text{bottom}) / (1 + 10^{(\log EC_{50} - x)})]$, where top and bottom are the percentage of 120 mM KCl maximum contractile response at the plateaus, and the EC50 is the molar concentration of alkaloid producing 50% of the KCl maximum response. Data without regression lines signifies the data could not be fit to the sigmoidal concentration response model.

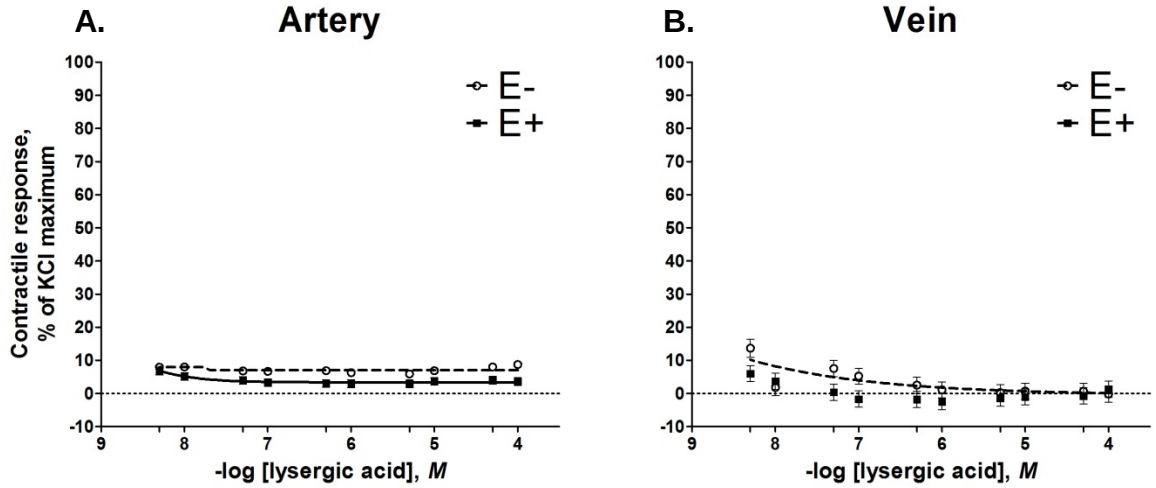


Figure 3.7. Mean contractile response of mesenteric artery (A) and vein (B) to increasing concentrations of an ergovaline-containing extract (**EXT**) for endophyte-infected (**E+**) and endophyte-free (**E-**) tall fescue seed-treated steers. A) For artery, effects of seed, concentration, and seed × concentration were significant ($P < 0.05$). B) For vein, effects of concentration and seed × concentration were significant ($P < 0.05$), yet the main effect of seed was not ($P = 0.40$). Regression lines shown are due to using non-linear regression analysis to fit the data to a sigmoidal concentration response curve which utilized the following equation: $y = \text{bottom} + [(\text{top} - \text{bottom}) / (1 + 10^{(\log EC_{50} - x)})]$, where top and bottom are the percentage of 120 mM KCl maximum contractile response at the plateaus, and the EC50 is the molar concentration of alkaloid producing 50% of the KCl maximum response.

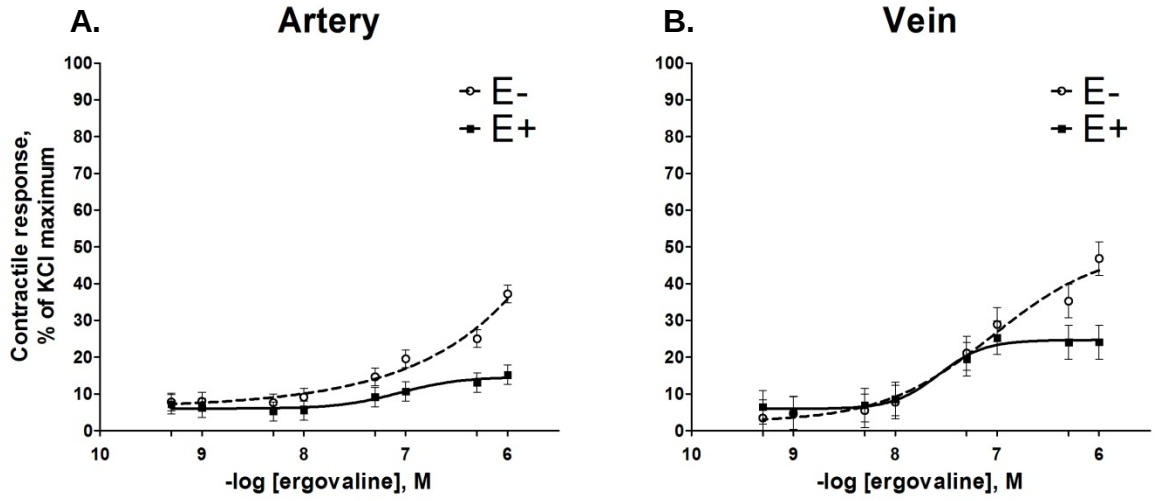
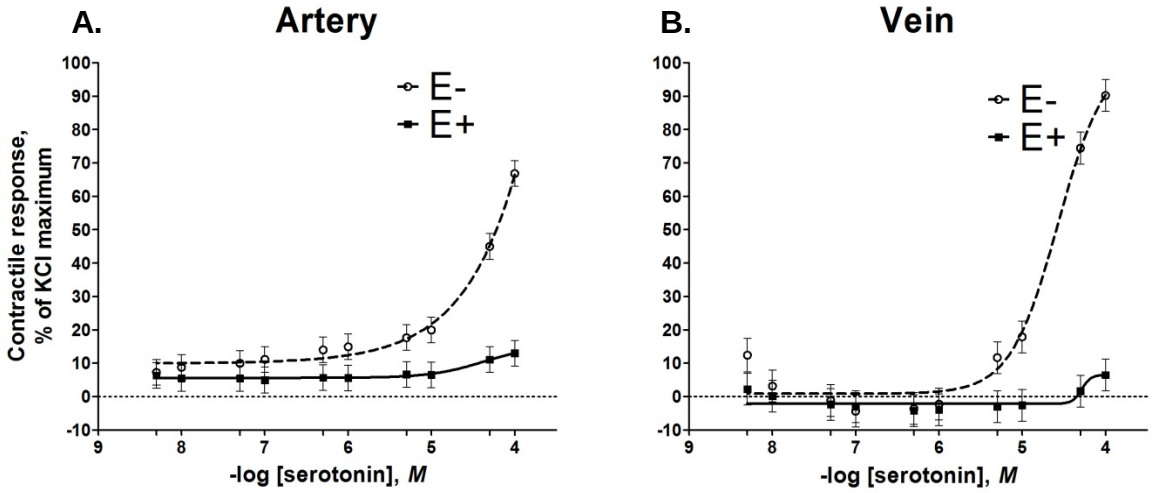


Figure 3.8. Mean contractile response of mesenteric artery (A) and vein (B) to increasing concentrations of 5-hydroxytryptamine (**5HT**) for endophyte-infected (**E+**) and endophyte-free (**E-**) tall fescue seed-treated steers. A and B) The effects of seed, concentration, and seed × concentration were significant ($P < 0.01$). Regression lines shown are due to using non-linear regression analysis to fit the data to a sigmoidal concentration response curve which utilized the following equation: $y = \text{bottom} + [(\text{top} - \text{bottom}) / (1 + 10(\log EC50 - x))]$, where top and bottom are the percentage of 120 mM KCl maximum contractile response at the plateaus, and the EC50 is the molar concentration of alkaloid producing 50% of the KCl maximum response.



Copyright © Journal of Animal Science 2014

Chapter 4: Development of a methodology to measure the effect of ergot alkaloids on forestomach motility using real-time wireless telemetry[2]

Introduction

Numerous factors affect motility of the reticulorumen including diet composition, feed and water intake, environmental temperature, feeding versus resting activity, volatile fatty acid concentrations, and metabolic conditions, such as hypocalcemia, as well as individual animal variation (Church, 1976). Additionally, many methods have previously been used for measuring forestomach motility, such as electromyography (McLeay and Smith, 2006; Poole et al., 2009), radiotelemetry (Cook et al., 1986), and pressure-sensitive recordings of ruminal gas (Colvin and Daniels, 1965) or fluids (Dado and Allen, 1993). In order to adequately evaluate the effect of a treatment on rumen motility, one must first understand typical rumen motility patterns throughout the entire feeding cycle.

Ergot alkaloids, which are produced by a symbiotic endophyte associated with tall fescue grass (Lyons et al., 1986), cause fescue toxicosis in grazing livestock (Strickland et al., 2011). Fescue toxicosis syndrome can be costly for livestock producers due to decreased average daily gains, feed intake, milk production, and conception rates (Strickland et al., 2011). Westendorf et al. (1993) found that about 93-96% of ergot alkaloids consumed are absorbed or transformed in the gastrointestinal tract. Additionally, it was determined that only 50 - 60% of the ergot alkaloids administered in the diet are recovered in the abomasum, which means that a large

[2] Published with permission from Frontiers in Chemistry
Egert, A. M., J. L. Klotz, K. R. McLeod, and D. L. Harmon. 2014b. Development of a methodology to measure the effect of ergot alkaloids on forestomach motility using real-time wireless telemetry. Front. Chem. DOI: 10.3389/fchem.2014.00090.

portion (40 - 50%) of ergot alkaloids in the diet are metabolized or absorbed in the forestomach.

Recent research with ergot alkaloids has suggested that rumen motility may also be altered with endophyte-infected tall fescue consumption. For example, Foote et al. (2013) demonstrated that the DM percentage and dry contents of the rumen on a BW basis were greater for cattle that were ruminally dosed with endophyte-infected (**E+**) tall fescue seed compared to cattle dosed with endophyte-free (**E-**) seed. This finding could indicate a difference in particulate or liquid passage rates. One hypothesis is that reduced passage rate in E+ steers could be a result of decreased rumen motility. Ergot alkaloids, specifically ergotamine and ergovaline, have been shown to decrease contractions and increase baseline tonus of reticulorumen smooth muscle in sheep when administered intravenously (McLeay and Smith, 2006; Poole et al., 2009). Yet, there has not been research investigating the effect of ergot alkaloids or endophyte-infected tall fescue seed on rumen motility patterns in cattle. Furthermore, data on ruminal or oral dosing of ergot alkaloids and the effect on rumen motility is lacking.

Therefore, the objectives of Exp. 1 were to characterize rumen motility patterns relative to feeding using a pressure transducer and real-time, wireless telemetry system and determine when, relative to feeding, to measure motility. Using the time period as determined in Exp. 1, the objective of Exp. 2 was to investigate the effects of ruminal dosing of endophyte-infected tall fescue seed on rumen motility, rumen dry matter contents, and ruminal fill in cattle.

Materials and Methods

The procedures used in this study were approved by the University of Kentucky Institutional Animal Care and Use Committee. Experiments were conducted at the University of Kentucky C. Oran Little Research Center in Woodford County.

Experiment 1

Animal Management

Eight ruminally cannulated Holstein steers (BW = 321 ± 11 kg) were fed alfalfa cubes (% composition on a DM basis: CP = 16.8; ADF = 33.5; NDF = 43.1; NFC = 29.1; TDN = 59; NE_m = 5.09 MJ/kg) at 1.5× NE_m once daily (0830 h) top-dressed with a trace mineral pre-mix (Kentucky Nutrition Service, Lawrenceburg, KY, USA; NaCl = 92-96%; Fe = 9275 ppm; Zn = 5500 ppm; Mn = 4790 ppm; Cu = 1835 ppm; I = 115 ppm; Se = 18 ppm; Co = 65 ppm) to meet or exceed nutrient requirements (NRC, 2000). Steers were housed indoors at 22°C in individual 3 × 3 m stalls and given ad libitum access to water.

Telemetry System

A wireless telemetry system (emkaPACK*4G* telemetry system, emka TECHNOLOGIES USA, Falls Church, Virginia) was used to monitor real-time pressure changes in the rumen. The system consisted of 2 receivers, 8 transmitters, and 8 bptVAP modules (pressure transducers). Wireless receivers were mounted securely to the wall outside of the pens in the room with the steers. The receivers were hardwired to a POE+ switch (8-port gigabit GREENnet POE+ switch, TRENDnet, Torrance, CA) that

was connected to a laptop. All cable connections were made using E5 ethernet cables. Transducers were connected to their corresponding transmitters using the auxiliary port. During experimentation, the transducer and transmitter were housed in a 1 L cylindrical plastic container with screw-on lid (Gordon Food Service, Wyoming, MI), which replaced the cap in the cannula opening. A stainless steel female luer lock bulkhead adapter inserted into the bottom of the container served as the connection between the transducer and catheter. A female luer lock to 2.4 mm barb adapter connected the transducer to a 5.5 cm piece of silicone tubing (i.d. = 2.4 mm; o.d. = 4.0 mm) attached to the barb of the bulkhead adapter. The transducer was taped to the side of the container to prevent kinks in the tubing.

The catheter was a section of 96.5 cm long Tygon tubing (i.d. = 3.2 mm; o.d. = 6.4 mm) with 8 fused Tygon tubing cuffs and a 22.9 cm latex balloon (Bargain Balloons, Niagara Falls, NY) on one end, which was prefilled with 1 L of water. The cuffs enabled consistent placement of the balloons on the end of the catheter. On the other end of the catheter, a 3-way stopcock was connected by means of a female luer lock to 3.2 mm barb adapter. The catheter was weighted with approximately 300 g anchored approximately 4 cm from the top of the balloon. Balloons, which were replaced before each data collection period, were secured to the catheter using latex castration bands (Ideal Instruments, Neogen Corporation, Lansing, MI) placed over the balloon tongue and clamped tightly onto the catheter by plastic hose clamps (acetyl copolymer; i.d. minimum = 11.4 mm; i.d. maximum = 13 mm; Cole-Palmer Instrument Co., Vernon Hills, IL). Cheesecloth was placed into the container to prevent excessive movement of the transmitter. Upon submerging the water-filled balloon in the ventral sac of the rumen, the attached container was inserted into the cannula opening. A piece of nylon webbing over the lid of the container was secured to the cannula with nylon screws and thumb nuts (1/4-20; Non-Ferrous Fastener Inc., Chino, CA) to keep the container in place.

Signal Calibration

Each transmitter and pressure transducer combination was manually calibrated using a sphygmomanometer connected to the “out” port of the transducer. Calibration was performed in the two-points (sampled) mode (20 and 200 mm Hg) of iox2 software (iox 2.9.4.27, emka TECHNOLOGIES USA) before data collection commenced on each day. To verify that calibration was successful, various amounts of pressure were applied with the pressure gauge to check that values on the gauge matched values in iox2 software (emka TECHNOLOGIES USA).

Data Collection and Analysis

Data were collected for 24 consecutive hours to capture the entire feeding cycle on three separate instances for each steer. Collection periods began, immediately following feeding, at 0830 and ended at 0830 the following day. Data were recorded and stored using iox2 software (emka TECHNOLOGIES USA) with a sampling rate of 100 pressure readings per second. Smoothing was set to 20 samples (200 ms) to help eliminate some background and movement noise in the signal.

The rhythmic analyzer in iox2 was used simultaneously while data were collected to analyze the raw rumen pressure data, identify ruminal contractions, and calculate the following parameters for each contraction: baseline, peak, amplitude, frequency, time to peak (TTP), relaxation time (RT), and area under the curve. The log and storage cadence was set to event related mode calculating the mean of these parameters for each event. By definition, for data to be considered an event or contraction, the signal must have increased at least 4 mm Hg from baseline (event threshold). Additionally, the

contraction must have a slope value for the TTP start threshold of at least 0.500 mm Hg/sec.

Individual water intake was also recorded using water meters every 8 h after feeding on collection days.

Statistical Analysis

Statistical Analysis Systems software (SAS; SAS Inst. Inc., Cary, NC) was used to calculate duration (TTP + RT) of each contraction. Values for baseline, peak, amplitude, frequency, TTP, RT, duration, and area for each animal were averaged for each hour after data collection began using the Proc Means procedure of SAS 9.3. Hourly means for the above variables were analyzed using a Proc GLIMMIX with repeated measures model of SAS considering animal as a random effect and effect of hour and thirds (consecutive 8-h periods within d, i.e. h 1 - 8, 9 – 16, and 17 – 24) as fixed effects. Tests for differences between days for each variable were conducted using contrasts. The Proc TTEST of SAS was run to determine equality of variances between thirds of the day after feeding for amplitude, frequency, duration, and area.

Experiment 2

Animals and Treatments

The same eight ruminally-cannulated Holstein steers (BW = 378 ± 12 kg) used in Exp. 1 were paired by weight in a randomized complete block design. Within block, one steer was assigned to each treatment: E- (“KY 32”; 0 mg ergovaline + ergovalinine / kg DM) or E+ (“KY 31”; 2.87 mg ergovaline + ergovalinine / kg DM; 0.65 mg ergotamine +

ergotaminine / kg DM) tall fescue seed treatment. Tall fescue seed was analyzed for ergovaline isomer and ergotamine isomer concentrations using a HPLC with fluorescence detection procedure modified from Yates and Powell (1988). Steers were pair-fed the basal diet in Exp. 1 once daily (0800 h) starting at 1.5 × NEm (NRC, 2000). Thus, the E- steers only received the quantity of feed their paired E+ steer consumed the previous day. Tall fescue seed was ground by a grinder mixer (MX125, Gehl, West Bend, WI) to pass through a 3-mm screen. Immediately before feeding, all steers were dosed with 1.45 kg tall fescue seed through the cannula opening for 15 d. The E+ steers received 10 µg ergovaline + ergovalinine / kg BW. Therefore, a combination of E+ and E- seed was used to achieve this dosage level for the E+ treatment animals. Steers were housed indoors at the University of Kentucky C. Oran Little Research Center in Woodford County at 22°C in individual 3 × 3 m stalls. Ad libitum access to water was provided.

Telemetry System and Signal Calibration

Experiment 2 utilized the wireless telemetry system (emka TECHNOLOGIES USA) and signal calibration procedure as described previously.

Data Collection and Analysis

During the first 14 d of ruminal seed dosing, an 8-h data collection period began 8 h after feeding (1600 h) each day. Data were recorded and stored using iox2 software with a sampling rate of 100 pressure readings per second with smoothing averaging every 20 readings (200 ms). Water intake was recorded using water flow meters

immediately after feeding, before collection, and after collection. The rhythmic analyzer in iox2 was used as it was in Exp. 1 for data analysis.

Blood Collection

On d 1, 7, and 15, blood was collected from the jugular vein immediately before seed dosing and feeding. Blood samples were allowed to clot for 24 h at 4°C and centrifuged at 1,500 x g for 25 min (4°C). Serum prolactin concentrations were analyzed by radioimmunoassay procedures of Bernard et al. (1993). The intraassay CV was 10.1% and the interassay CV was 7.4%.

Ruminal Evacuation

Complete manual evacuation of the rumen contents was conducted 8 h after feeding on d 15 through the cannula. Ruminal fill was measured for each steer by weighing total rumen contents. Three replicate samples (approximately 100 g each) were taken from the ruminal contents of each steer for DM analysis. The remaining contents were placed back into the rumen immediately after sampling.

Statistical Analysis

Calculations were as described for Exp. 1. Values for baseline pressure, peak pressure, amplitude, frequency, TTP, RT, duration, and area under the curve for each animal were averaged over the 8 h period every day using the Proc Means procedure of SAS. Motility variables were analyzed as a randomized block design (RBD) with repeated measures for the effects of seed, day, and the interaction of seed × day. Water

intake, DM intake, and ruminal content measures were analyzed as an RBD for the effect of seed. Serum prolactin was analyzed as a randomized block design with repeated measures for fixed effects of seed, day, and the interaction. Probability of Type I error less than 0.05 was considered significant.

Results

Experiment 1

Mean (± SEM) water intakes for the first (1 – 8 h), second (9 – 16 h), and third (17 - 24 h) 8-h periods of the day were 30.28 ± 2.00, 6.14 ± 0.72, and 0.18 ± 0.04 L, respectively. Table 4.1 displays the mean value for each rumen contraction variable measured and the range between animals.

Contraction amplitude was greatest around feeding time (Figure 4.1A). Frequency of ruminal contractions was greatest at feeding time and decreased thereafter until 22 h after feeding (Figure 4.1B). The duration of contractions gradually decreased and then increased slightly 2 h before the next feeding (Figure 4.1C). Area under the curve for contractions decreased as time from feeding increased (Figure 4.1D).

All motility variables differed ($P < 0.01$) by hour and period (divided into 3, 8-h periods) of the day. The effect of day was not significant for most variables. However, the mean frequency of day 3 was higher ($P = 0.03$) than day 2, and mean area of day 3 was greater ($P = 0.03$) than day 1. Variance of the second 8 h period of the day was less than ($P < 0.01$) the first and third for area and less than ($P < 0.05$) the third for amplitude, frequency, and duration.

Experiment 2

Mean water intake tended (P = 0.10) to be lower for E+ steers than for E- steers (16.27 ± 5.13 L and 28.86 ± 5.13 L, respectively) before data collection, meaning the 8-h period in between feeding and the start of data collection (Figure 4.2). There were no differences in water intakes between E- and E+ steers during data collection (E-: 6.54 ± 2.82 L; E+: 9.21 ± 2.82 L) or from the end of the data collection to feeding the next day (overnight; E-: 0.37 ± 0.35 L; E+: 1.10 ± 0.35 L).

Table 4.2 shows the mean results of rumen motility variables between E- and E+ treated steers. Pressure at the peak of the contractions was smaller (P = 0.04) for E+ steers. There was also a tendency for baseline pressure to be smaller (P = 0.06) in E+ steers. The effect of day was significant (P < 0.05) for baseline pressure, peak pressure, and frequency, while tending (P = 0.10) to be different for duration. Contraction frequency had a tendency (P = 0.10) for a seed × day interaction (Figure 4.3).

Serum prolactin was not different between seed treatments for any of the days (Figure 4.4). There was a large decrease in prolactin concentration between d 1 and d 7 of the trial for both treatment groups, although it was not significant. Comparison of d 7 and 15 showed relatively similar prolactin concentrations.

Table 4.3 displays the results of rumen evacuation and rumen content dry matter analysis. Particular consideration has been given to the relative DM intake around the time of evacuation between E- and E+ seed treated steers in an attempt to account for differences in rate of intake, despite the pair feeding situation. Dry matter intakes were not different (P = 0.71) between groups throughout the duration of the experiment due to the pair-feeding. Percent DM of ruminal contents, wet contents, and dry contents, did not

differ (P > 0.05) between E- and E+ steers. However, there was a tendency (P = 0.07) for the wet contents per 100 kg BW basis to be lower in E+ steers. For the 8 h immediately prior to evacuations on d 15, water consumption was not different (P = 0.13) between E- and E+ steers (27.89 ± 5.29 L and 16.66 ± 5.29 L, respectively.

Discussion

Experiment 1

This experiment was the first recorded adaptation of the emkaPACK4G wireless telemetry system for use in cattle. Most studies conducted with this system used canines (Bailey et al., 2011; McMahon et al., 2011) or non-human primates (Bruce et al., 2013). With the iox2 software, this system enables the measurement of many other variables beyond contraction amplitude and frequency, which are commonly the only variables reported as measurements of rumen motility (Attebery and Johnson, 1969; Bruce and Huber, 1973; Daniel, 1983; Cook et al., 1986). Additionally, other papers typically show values for these variables for the entire primary or secondary cycle (Froetschel et al., 1986; McSweeney et al., 1989; McLeay and Smith, 2006), whereas with this approach values for each contraction of the ventral sac are obtained. Because of this, it was difficult to find comparisons for some of these variables in published literature. The wireless aspect of this technology enables the animals to move freely and naturally in their environment. Moreover, the procedure for this technology is less invasive than other alternatives for measuring rumen motility, such as electromyography, and enables researchers to obtain a more detailed measurement of ruminal contractions.

Cannulation likely alters rumen motility and some measurements of motility may not be applicable to a non-cannulated animal. Mooney et al. (1971) found that cannulation decreased reticular contraction frequency during rest, but not during feeding or rumination. Also, amplitude of reticular contractions was significantly greater during feeding in intact animals. Frequency and amplitude of ruminal contractions within cannulated cattle also varies between sources. Attebery and Johnson (1969) reported frequencies between 1.74 and 2.23 contractions per min and amplitudes of 7.52 to 14.86 cm water in fed cows at various temperatures. However, observations were only taken for 30 min on 5 animals. Conversely, Daniel (1983) found an average frequency of the dorsal sac to be 1.13 ± 0.309 contractions per min and average amplitude of 14.7 ± 2.58 mm Hg prior to inducing hypocalcemia in cows. In this study, the mean frequency of ruminal contractions in steers was greater than those previously described, and the mean amplitude was generally lower. Differences could be attributable to the diet composition, method of measurement, time of recording relative to feeding, and length of recording.

Although statistical differences were found between certain days for frequency and area, graphically the days do not appear different. Therefore, these differences may not be physiologically relevant. Baseline and peak pressure displayed the greatest standard errors and ranges of all motility variables measured. This is likely due to the nature of the experiment and animal management as baseline and peak increase when the animal is laying down. Since the animals were allowed to move freely and stand up or lay down at will, the standard error of the mean and ranges for these parameters were more variable. Overall, small standard errors and ranges achieved here for measured parameters suggest that this approach to monitoring rumen motility is repeatable and consistent.

The second 8-h period of the day was the least variable for many measures of motility tested and had a moderate water intake. Therefore, it was concluded that measurements of motility for 9 – 16 h after feeding provide the best opportunity for testing differences in motility related to treatments because it provided the time when the pressure signal could be most consistently analyzed by the software due to less background noise. Feeding management will affect the values obtained and should be considered when designing experiments.

Experiment 2

Research on the effects of endophyte-infected tall fescue or ergot alkaloids on rumen motility has been minimal. Previous research has been done via electromyography in sheep utilizing the direct intravenous injection of ergotamine and ergovaline (McLeay and Smith, 2006; Poole et al., 2009). However, there are no published data on the effects in cattle. Additionally, the route of administration could have an impact on the effects. Ergot alkaloids injected directly into the blood stream might cause a greater degree of biological reaction, such as vasoconstriction, than ergot alkaloids consumed orally or given intra-ruminally. As a result, this study attempted to delineate two aspects of information that are lacking: 1) the effect of ergot alkaloids on rumen motility specifically in cattle and 2) the effect on rumen motility when ergot alkaloids are dosed intraruminally (as opposed to intravenously).

Similarities between the ergoline ring of ergot alkaloids and dopamine enables ergot alkaloids to bind D2-dopamine receptors (Berde and Stürmer 1978; Goldstein et al., 1980; Sibley and Creese, 1983). By binding to and activating these D2 receptors in the anterior pituitary gland, ergot alkaloids can inhibit the secretion of prolactin (Hurley et

al., 1980; Schillo et al., 1988; Porter and Thompson, 1992) through second messenger responses (Larson et al., 1995). As a result, reduced serum prolactin concentrations have been used as an indicator of fescue toxicosis, yet do not indicate severity by level of decrease. There are multiple reports of cattle consuming endophyte-infected tall fescue or seed where a depression in serum prolactin concentration was shown (Schillo et al., 1988; Klotz et al., 2012; Koontz et al., 2012; Foote et al., 2013).

In this study, there was a large numerical decrease in serum prolactin concentrations from the first day of seed dosing to mid experiment in both seed treatment groups. The mean prolactin concentration for E+ steers was lower than E- steers throughout the experiment, yet, results were not statistically different. Therefore, prolactin data do not support that these steers were experiencing acute fescue toxicosis. Although it was chosen as an intermediate dosage from published studies showing reduced serum prolactin concentrations in E+ treated steers, the dosage rate of 10 µg ergovaline + ergovalinine / kg BW may have been too small to induce fescue toxicosis under thermoneutral conditions. Kim et al. (2013) administered approximately 8 µg ergovaline + ergovalinine / kg BW, whereas Foote et al. (2013) dosed 15 µg ergovaline + ergovalinine / kg BW. Both of these experiments successfully induced fescue toxicosis and utilized ground endophyte-infected tall fescue seed given intraruminally at thermoneutral temperatures, as was done in this study.

In contrast, other signs of fescue toxicosis were demonstrated. Reductions in DM intake were observed for many E+ steers, and E+ steers routinely consumed their daily ration at a slower rate than E- steers. Similarly, Koontz et al. (2012) showed a greater rate of dry matter intake at thermoneutral conditions for E- steers. Dry matter intake rate could not be controlled in this study with once daily feeding. This may help explain the baseline and peak pressure of E+ steers being lower than E- steers. For

instance, if E- steers have consumed all of their feed, they would have likely been lying down more often during data collection, increasing the pressure, compared to the E+ steers, who still had food left to consume from their feed bunks. However, standing and laying behaviors were not monitored.

There was a tendency for a seed × day interaction for frequency of contractions (Figure 4.3). However, this is difficult to discern except that the E- steer contractions were less frequent than E+ steers on d 11.

There was also a tendency for greater water intake by E- steers compared to E+ steers during the first 8-h period following feeding, the period before motility data collection began. This was likely the result of the increased eating rate mentioned above and could have altered subsequent rumen motility (Church, 1976), although no significant differences were found. Aldrich et al. (1993) also reported water intake of steers was not changed with the consumption of tall fescue seed. Overall, the values obtained for rumen motility variables measured in this experiment agree with Experiment 1 and provide more support to the consistency of this approach.

Studies have demonstrated that cyclical contractions of reticuloruminal smooth muscle of sheep can be reduced or inhibited with the intravenous injection of ergot alkaloids (McLeay and Smith, 2006; Poole et al., 2009). This could potentially lead to a decreased passage rate of particulate or liquid matter, which could account for a reduction in intake as is commonly seen with ruminants experiencing fescue toxicosis. Unlike Foote et al. (2013), no differences were found in dry matter percentage of ruminal contents or dry contents (kg / 100 kg BW) between E+ and E- steers in this study. The lack of effect on ruminal dry matter contents is likely a result of the E+ seed treatment not effectively inducing fescue toxicosis. Additionally, differences may be due to the

time, relative to feeding, that rumen evacuations were conducted and rumen content samples were collected; Foote et al. (2013) gathered rumen content samples before feeding, whereas this study utilized rumen content samples collected 8 h after feeding.

Research has shown that duration of reticular contractions instead of frequency may have a larger influence on passage rate of ruminal fluid and particulate matter (Okine et al., 1989). The same theory could be applied to duration and frequency of ruminal contractions. However, seed treatment did not affect duration of contractions in this experiment.

Conclusions

The emkaPACK4G wireless telemetry system can be used as an accurate, effective, and non-invasive tool to measure rumen motility and obtain detailed measurements of ruminal contractions in ruminally cannulated animals. Endophyte-infected tall fescue seed treatment at a dosage of 10 µg ergovaline + ergovalinine / kg BW under thermoneutral conditions for 14 days (which failed to induce acute fescue toxicosis) did not significantly alter rumen motility, ruminal fill, or dry matter of rumen contents. Therefore, it remains unclear as to whether ergot alkaloids or endophyte-infected tall fescue dosed intraruminally decreases rumen motility. Future experiments should focus on the interactions of ergot alkaloid dosage, ambient temperature, intake and feeding behavior, and rumen motility.

Acknowledgements

The authors acknowledge Lauren Clark of the University of Kentucky C. Oran Little Research Center Beef Unit for her hard work in helping conduct these experiments and Edward Roualdes for statistical consulting for Experiment 1.

Table 4.1. Mean values and range between animals for rumen contraction variables.

Item, units	Mean[1]	SEM[2]	Range[3]
Baseline, mmHg	22.98	2.68	7.28
Peak, mmHg	30.28	2.78	7.29
Amplitude, mmHg	7.29	0.46	1.07
Frequency, contractions/min	2.87	0.23	0.92
Time to peak, s	4.06	0.42	0.85
Relaxation time, s	5.23	0.50	1.04
Duration, s	9.29	0.73	1.34
Area, mmHg*s	30.47	2.99	6.93

[1] Mean = overall mean, n = 576
[2] SEM = standard error of the mean, n = 8
[3] Range = range of means among the 8 steers

Table 4.2. Mean results for rumen motility contraction variables measured for 14 days in E- and E+ tall fescue seed treated steers.

Item	Seed Treatment		SEM	*P*-values		
	E-[1]	E+[2]		Seed	Day	Seed × Day
Baseline, mm Hg	29.73	27.11	0.81	0.06	< 0.01	0.43
Peak, mm Hg	36.68	34.30	0.65	0.04	< 0.01	0.29
Amplitude, mm Hg	6.95	7.20	0.28	0.55	0.46	0.24
Frequency, contractions/min	2.95	3.02	0.12	0.68	0.03	0.10
Time to peak, s	4.29	4.43	0.11	0.43	0.35	0.39
Relaxation time, s	4.98	4.96	0.19	0.90	0.10	0.28
Duration, s	9.29	9.38	0.20	0.50	0.10	0.78
Area, mm Hg*s	28.86	31.55	1.91	0.36	0.13	0.84

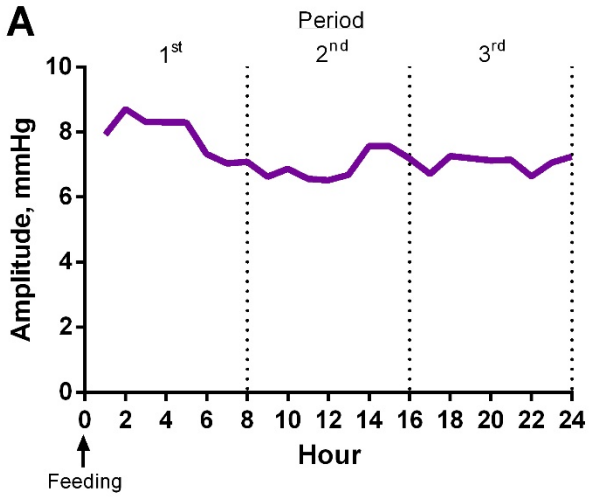
[1] E- = endophyte-free tall fescue seed
[2] E+ = endophyte-infected tall fescue seed

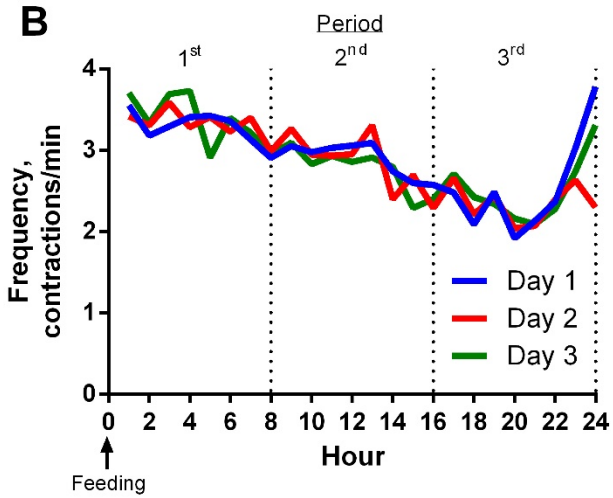
Table 4.3. Dry matter intakes and ruminal contents measured by rumen evacuations on d 15 and DM analysis.

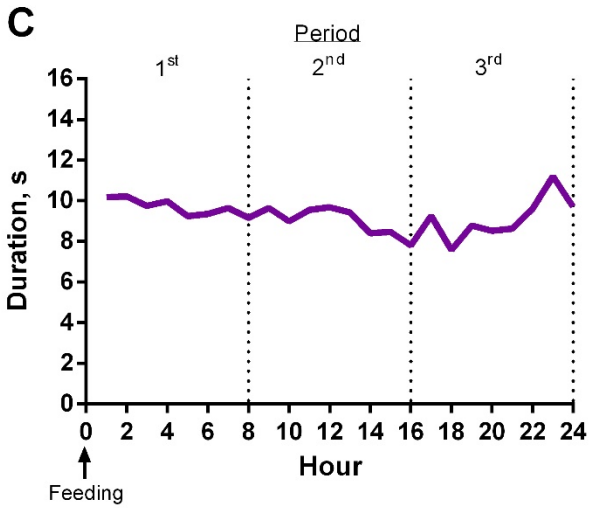
Item	Seed Treatment		SEM	*P*-value
	E-	E+		
Intakes				
DM[1], kg	9.13	9.12	0.18	0.71
DM 8 h before evacuations, kg	9.84	7.59	1.02	0.22
DM 32 h before evacuations, kg	20.37	17.93	1.10	0.22
Ruminal Contents				
Percent DM	15.58	16.37	0.55	0.39
Wet contents, kg	66.50	59.68	3.47	0.16
Wet contents, kg/ 100 kg BW	17.58	15.80	0.47	0.07
Dry contents, kg	10.32	9.77	0.55	0.35
Dry contents, kg/ 100 kg BW	2.75	2.58	0.08	0.22
Dry contents, % of intake last 8 h	105.27	143.59	20.46	0.25
Dry contents, % of intake last 32 h	50.63	55.60	4.11	0.37

[1] Mean DM intake for d 1 through d 14 with pair-feeding management

Figure 4.1. Experiment 1 Results. **A)** Mean contraction amplitude of steers (n = 8) for each hour relative to feeding. The mean contraction amplitude of the first 8 h period was higher ($P < 0.01$) than the second. The mean contraction amplitude of the second 8 h period was not different ($P = 0.96$) from the third. **B)** Mean contraction frequency of steers (n = 8) for each hour relative to feeding. Mean contraction frequency of day 2 differed ($P = 0.03$) from day 3. The mean contraction frequency of the first 8 h period was higher ($P < 0.01$) than the second. The mean contraction frequency of the second 8 h period was different ($P < 0.01$) from the third. Additionally, the average of the first and third 8 h periods was not different ($P = 0.35$) from the second. **C)** Mean contraction duration of steers (n = 8) for each hour relative to feeding. The mean contraction duration of the first 8 h period was longer ($P < 0.01$) than the second. The mean contraction duration of the second 8 h period was not different ($P = 0.34$) from the third. **D)** Mean contraction area of steers (n = 8) for each hour relative to feeding. Mean contraction area of day 1 differed ($P = 0.03$) from day 3. The mean contraction area of the first 8 h period was greater ($P < 0.01$) than the second. The mean contraction area of the second 8 h period tended ($P = 0.07$) to be greater than the third.


A
Period
1st
2nd
3rd
Amplitude, mmHg
0
2
4
6
8
10
0 2 4 6 8 10 12 14 16 18 20 22 24
Hour
Feeding


B
Period
1st
2nd
3rd
Frequency, contractions/min
0
1
2
3
4
0 2 4 6 8 10 12 14 16 18 20 22 24
Hour
Feeding
Day 1
Day 2
Day 3


C
Period
1st
2nd
3rd
Duration, s
0
2
4
6
8
10
12
14
16
0 2 4 6 8 10 12 14 16 18 20 22 24
Hour
Feeding

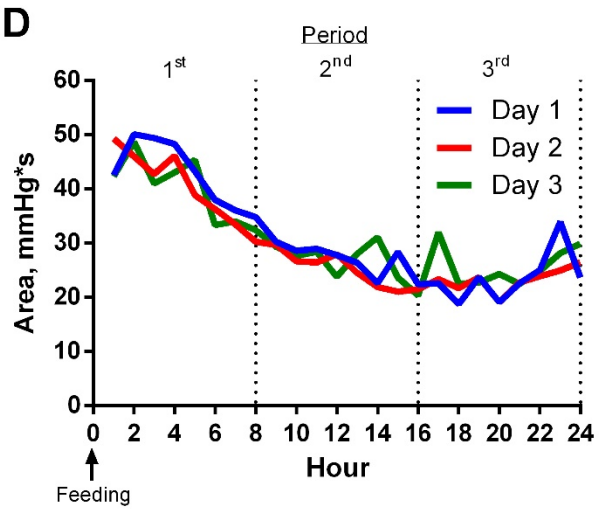

D
Period
1st
2nd
3rd
Area, mmHg*s
0
10
20
30
40
50
60
0 2 4 6 8 10 12 14 16 18 20 22 24
Hour
Feeding
Day 1
Day 2
Day 3

Figure 4.2. Water intake by time period, relative to data collection, for endophyte-free (E-) and endophyte-infected (E+) tall fescue seed treated steers. Steers in the E+ treatment group received 10 µg ergovaline + ergovalinine / kg BW daily. In the 8 h immediately before data collection commenced, water intake tended (P = 0. 10) to be greater for E- steers. Water intake was not different between seed treatment groups during data collection (P = 0.55) or overnight (P = 0.23).

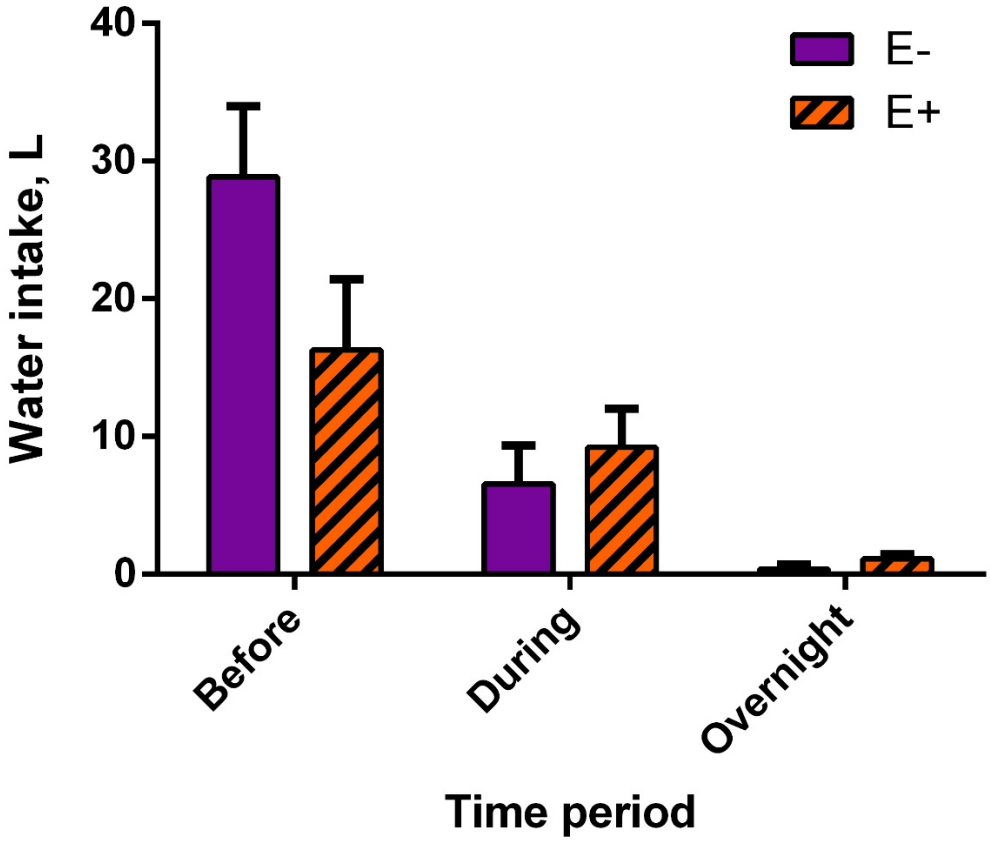


Figure 4.3. Frequency of contractions for endophyte-free (E-) and endophyte-infected (E+) tall fescue seed treated steers each day of the experiment. Steers in the E+ treatment group received 10 µg ergovaline + ergovalinine / kg BW daily. The effect of day was significant (P = 0.03), and there was a tendency (P = 0.10) for a seed × day interaction.

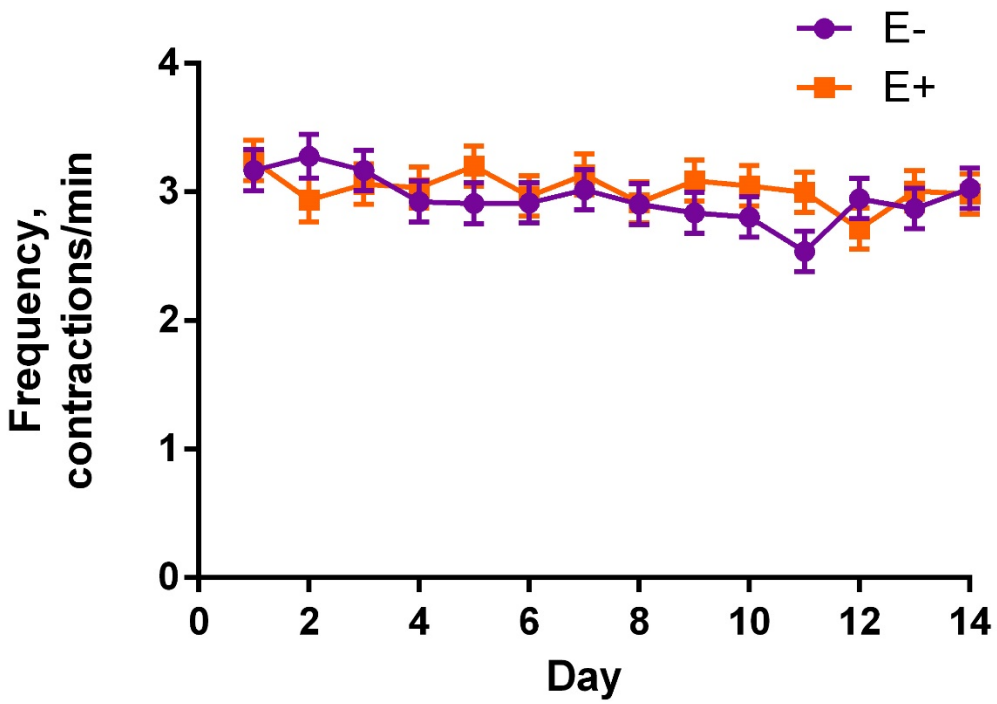
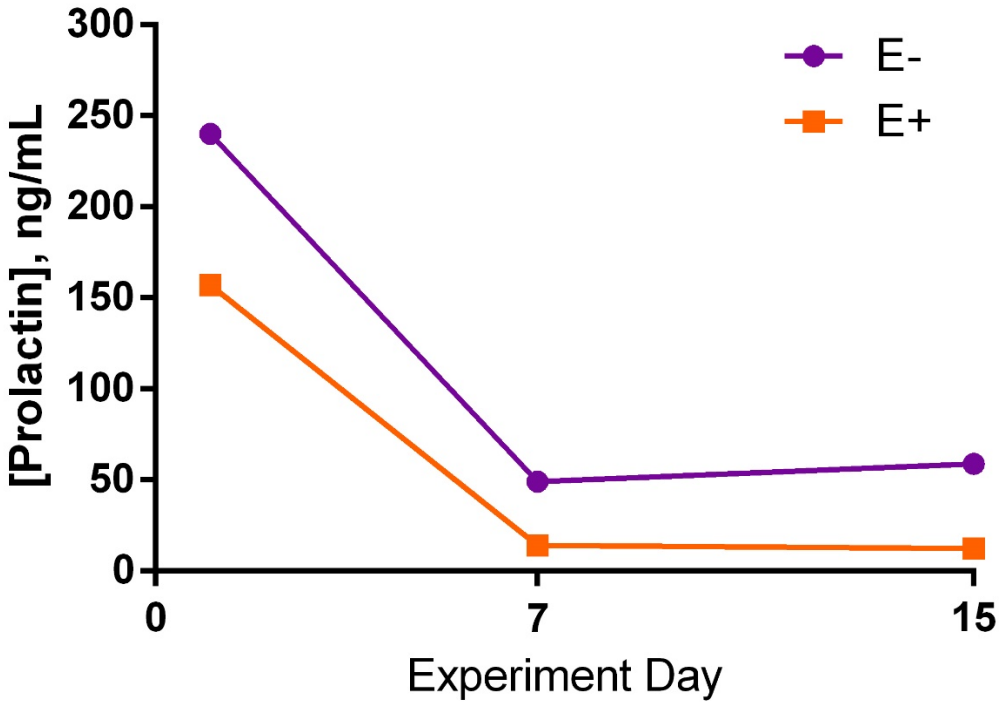


Figure 4.4. Serum prolactin concentrations (SEM = 50.4) for endophyte-free (E-) and endophyte-infected (E+) seed-treated steers throughout the experiment. Steers in the E+ treatment group received 10 µg ergovaline + ergovalinine / kg BW daily. The effects of seed, day, and the interaction were not significant (P > 0.05).



Copyright © Frontiers in Chemistry 2014

Chapter 5: Summary and Conclusions

Association of *Neotyphodium coenophialum*, a symbiotic endophyte, with tall fescue, a prominent cool season grass, makes wild-type endophyte-infected tall fescue a resilient forage for animal production systems, particularly in the transition zone of the Southeast United States (Porter et al., 1979; Bush et al., 1982; Bacon and Siegel, 1988). The fungal endophyte confers desirable properties for the growth, persistence, and maintenance of the plant, making it a valuable pasture grass which can handle heavy grazing. However, it is now known that the endophyte produces compounds which cause fescue toxicosis in animals that consume endophyte-infected tall fescue grass, hay, or seed (Lyons et al., 1986; Strickland et al., 2011). Fescue toxicosis results in decreased animal performance as evidenced by reduced gains, intake, reproduction efficiency, serum prolactin, etc. The production losses related to wild-type endophyte-infected tall fescue consumption are significant and are of particular concern for cow-calf and backgrounding cattle operations across the United States, which utilize the majority of endophyte-infected tall fescue acreage.

Ergot alkaloids are the principle compounds implicated in the causation of fescue toxicosis. These compounds are created by the fungus and have been well documented in literature as vasoactive agents in cattle and other mammals, likely due to their structural similarities with neurotransmitters. Ergot alkaloids induce vasoconstriction in a variety of bovine vasculature types (Rhodes et al., 1991; Solomons et al., 1989; Klotz et al., 2006; Aiken et al., 2007; Foote et al., 2011), which could be related to some symptoms of fescue toxicosis. Additionally, blood flow to GIT tissues, such as the rumen, duodenum, and colon, have been found to decrease with endophyte-infected tall fescue consumption (Foote et al., 2013; Rhodes et al., 1991). Vasoconstriction of blood

vessels supporting and exiting GIT tissues may affect nutrient absorption and therefore, availability. As a result, this may be a potential reason why cattle consuming endophyte-infected tall fescue display poor growth.

While flux of nutrients was not measured, the first experiment in this series was the first to demonstrate that ergot alkaloids do cause vasoconstriction of bovine mesenteric vasculature in vitro to various degrees depending on the specific ergot alkaloid. Ergovaline was a very potent vasoconstrictor as seen in many other studies. Steers that were dosed ruminally with endophyte-infected tall fescue seed exhibited smaller i.d. and o.d. of mesenteric artery compared to endophyte-free seed treated cattle. Thus, vasoconstriction in vivo is also evident. Lysergic acid failed to produce a contractile response in mesenteric vasculature, whereas several ergot alkaloids caused vasoconstriction, which further potentiates the suggestion that ergot alkaloids are the main causative agents involved in fescue toxicosis. Additionally, vessels from E+ steers exhibited weaker contractile responses to ergot alkaloids incubated in vitro, demonstrating that immediate prior exposure to ergot alkaloids affects their vasoactivity. However, the removal of the endophyte-infected diet for a period of time may diminish this effect as the mechanism of reduced vasoactivity with prior exposure is not fully understood.

Historically, rumen motility has previously been measured with a variety of different methods making it difficult to compare between studies. Experiment two of this series developed a non-invasive method of measuring rumen motility in ruminally-cannulated cattle and determined that 8 to 16 h after feeding provided a valid representation of rumen motility with the least amount of variation due to individuals.

Ergot alkaloids associated with endophyte-infected tall fescue may interact with the sophisticated neuro-control of forestomach motility patterns and may alter rumen motility. Previous studies showed that intravenous injection of ergot alkaloids, specifically ergotamine and ergovaline, into sheep decreased forestomach motility as measured with electromyography (McLeay and Smith, 2006; Poole et al., 2009). The rumen is proposed to be the primary site for ergot alkaloid metabolism and absorption. Therefore, it was suggested that ergot alkaloids in the diet may decrease rumen motility. The third experiment in this series was the first to investigate the effect of ergot alkaloids dosed ruminally on rumen motility. Unfortunately, the experimental endophyte-infected tall fescue seed treatment did not effectively induce fescue toxicosis as evidenced by no difference in serum prolactin between E+ and E- seed-treated steers. No significant differences were found in rumen motility between treatments. Therefore, more remains to be investigated to determine the effect, if any, of ruminally dosed ergot alkaloids on rumen motility.

Future research should be directed towards characterization of the mechanisms by which ergot alkaloids induce vasoconstriction and alter rumen motility, metabolism of ergot alkaloids in the rumen, and effects of ergot alkaloids on nutrient absorption. Understanding the mechanisms by which ergot alkaloids cause fescue toxicosis will begin to allow for scientists to develop methods for the alleviation of this costly syndrome and improve animal health.

Copyright © Amanda M. Egert 2014

APPENDIX A: Permission to use material published in the Journal of Animal Science

October 8, 2014

Dr. Greg S. Lewis
Editor-in-Chief of the Journal of Animal Science
PO Box 7410
Champaign, IL 61826

Dear Dr. Lewis,

In partial fulfillment of the requirements for my M.S. thesis at the University of Kentucky, I have published the following paper in the Journal of Animal Science:

Egert, A. M., D. H. Kim, F. N. Schrick, D. L. Harmon, and J. L. Klotz. 2014. Dietary exposure to ergot alkaloids decreases contractility of bovine mesenteric vasculature. J. Anim. Sci. 92:1768-1799.

I request permission to use this paper, with appropriate copyright information and citation noted, in my thesis.

Thank you for your time and consideration,

Approved: Gregory S. Lewis
December 7, 2014

APPENDIX B: Permission to use material published in Frontiers in Chemistry journal

October 8, 2014

Dr. Steven L. Suib
Frontiers in Chemistry – Field Chief Editor
The University of Connecticut
Department of Chemistry, Unit 3060
55 North Eagleville Road
Storrs, CT 06269

Dear Dr. Suib,

In partial fulfillment of the requirements for my M.S. thesis at the University of Kentucky, I have published the following paper in Frontiers in Chemistry under the research topic "Recent Investigations of Ergot Alkaloids Incorporated into Plant and/or Animal Systems":

Egert, A. M., J. L. Klotz, K. R. McLeod, and D. L. Harmon. 2014. Development of a methodology to measure the effect of ergot alkaloids on forestomach motility using real-time wireless telemetry. Front. Chem. DOI: 10.3389/fchem.2014.00090.

The article was submitted on July 14th, 2014, has completed the review process, was accepted for publication on September 25th, 2014, and is waiting on final approval of author proof revisions. Dr. Darrin Smith is the editor of this section.

I request permission to use this article, with appropriate copyright information and citation noted, in my thesis.

Thank you for your time and consideration,

Amanda M. Egert

Suib, Steven <steven.suib@uconn.edu>
Wed, Oct 8, 2014 at 2:19 PM
To: "a.egert@uky.edu" <a.egert@uky.edu>
Cc: "Frontiers Research Topics Chemistry (chemistry.researchtopics@frontiersin.org)" <chemistry.researchtopics@frontiersin.org>
Marie,
Who can make a decision about granting permission to Dr. Egert?
Steve

Steven L. Suib
Board of Trustees Distinguished Professor
Director, Institute of Materials Science
860-486-2797; 2981 (FAX)
Steven.Suib@uconn.edu
http://chemistry.uconn.edu/SuibGroup/firstpage.htm

Frontiers Research Topics Chemistry <chemistry.researchtopics@frontiersin.org>
Thu, Oct 9, 2014 at 7:42 AM
To: a.egert@uky.edu

Dear Amanda Egert

Many thanks for your email and letter requesting to use material that you published in Frontiers in Chemistry.

Please be aware that under the Frontiers Terms and Conditions, authors retain the copyright to their work. All Frontiers articles are Open Access and distributed under the terms of the Creative Commons Attribution License, (CC-BY 3,0), which permits the use, distribution and reproduction of material from published articles, provided the original authors and source are credited, and subject to any copyright notices concerning any third-party content.

Therefore to use your publication in your M.S. thesis no permission is needed. Nevertheless, the full citation of the original publication is mandatory. Please follow the following example: "Figure as originally published in Weiss SA and Faber DS (2010) Field effects in the CNS play functional roles Front. Neural Circuits 4:15. doi: 10.3389/fncir.2010.00015".

Do not hesitate to contact me if you have any questions about this.

Kind regards,

Laura Smart

Frontiers | Chemistry Research Topics Editorial Office
Journal Manager: Dr Marie Soulière

EPFL Innovation Park - Building I
1015 Lausanne, Switzerland | T +41(0)21 510 17 34
www.frontiersin.org | twitter.com/FrontiersIn

For technical issues, please contact our IT Helpdesk - support@frontiersin.org

LITERATURE CITED

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

Aiken, G. E., B. H. Kirch, J. R. Strickland, L. P. Bush, M. L. Looper, and F. N. Schrick. 2007. Hemodynamic responses of the caudal artery to toxic tall fescue in beef heifers. J. Anim. Sci. 85:2337-2345.

Aldrich, C. G., K. N. Grigsby, J. A. Paterson, and M. S. Kerley. 1990. Performance, OM intake, and digestibility by steers when rotationally grazed from tall fescue pastures to warm-season annual grass pastures. J. Anim. Sci. 68 (Suppl. 1):559 (Abstr.).

Aldrich, C. G., J. A. Paterson, J. L. Tate, and M. S. Kerley. 1993a. The effects of endophyte-infected tall fescue consumption on diet utilization and thermal regulation in cattle. J. Anim. Sci. 71:164-170.

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

Attebery, J. T. and H. D. Johnson. 1969. Effects of environmental temperature, controlled feeding, and fasting on rumen motility. J. Anim. Sci. 29:734-737.

Arechevaleta, M., C. W. Bacon, C. S. Hoveland, and D. E. Radcliffe. 1989. Effect of tall fescue endophyte on plant response to environmental stress. Agron. J. 81:83-90.

Arechavaleta, M., C. W. Bacon, R. D. Plattner, C. S. Hoveland, and D. E. Radcliffe. 1992. Accumulation of alkaloids in symbiotic tall fescue grown under deficits of soil water and nitrogen fertilizer. Appl. Environ. Microbiol. 58:857-861.

Ash, R. W. 1959. Inhibition and excitation of reticulo-rumen contractions following the introduction of acids into the rumen and absomasum. J. Physiol. 147:58-73.

Ayers, A. W., N. S. Hill, G. E. Rottinghaus, J. A. Stuedemann, F. N. Thompson, P. T. Purinton, D. H. Seman, D. L. Dawe, A. H. Parks, and D. Ensley. 2009. Ruminal metabolism and transport of tall fescue ergot alkaloids. Crop Sci. 49:2309-2316.

Bacetty, A. A., M. E. Snook. A. E. Glenn. J. P. Noe, N. Hill, A. Culbreath, P. Timper, P. Nagabhyru, and C. W. Bacon. 2009. Toxicity of endophyte-infected tall fescue alkaloids and grass metabolites on *Pratylenchus scribneri*. Phytopathology 99:1336-1345

Bacon, C. W., J. K. Porter, J. D. Robbins, and E. S. Luttrell. 1977. *Epicholë typhina* from toxic tall fescue grasses. Appl. Environ. Microbiol. 34:576-581.

Bacon, C. W., P. C. Lyons, J. K. Porter, and J. D. Robbins. 1986. Ergot toxicity from endophyte-infected grasses: A review. Agron. J. 78:106.

Bacon, C. W. 1988. Procedure for isolating the endophyte from tall fescue and screening isolates for ergot alkaloids. Appl. Eviron. Microbiol. 54:2615-2618.

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

Bacon, C. W. 1993. Abiotic stress tolerances (moisture, nutrients) and photosynthesis in endophyte-infected tall fescue. Agric., Eco., and Environ. 44:123-141.

Bailey, E. C. Grant, J. Moors, J-L. Wang, J-G. Napoléoni, L. Ewart, P. Lainee, and J-P. Valentin. 2011. Simultaneous assessment of blood pressure and respiratory function in dogs: validation of a non-invasive telemetry method. Poster presented by AstraZeneca at the 11th Annual Meeting of the Safety Pharmacology Society, Innsbruck, Austria.

Balch, C. C., S. Bartlett and V. W. Johnson. 1951. Apparatus for the separate collection of feces and urine from cows. J. Agr. Sci. 41:98-101.

Ball, O. J. P., T. A. Coudron, B. A. Trapper, E. Davies, D. Trently, L. P. Bush, K. D. Gwinn, and A. J. Popay. 2006. Importance of host plant species, *Neotyphodium* endophyte isolate, and alkaloids on feeding by *Spodoptera frugiperda (*Lepidoptera: Noctuidae) larvae. J. Econ. Entomol. 99:1462-1473.

Belesky, D. P., J. A. Stuedemann, R. D. Plattner, and S. R. Wilkinson. 1988. Ergopeptine alkaloids in grazed tall fescue. Agron. J. 80:209-212.

Berde, B., and E. Stürmer. 1978. Introduction to the pharmacology of ergot alkaloids and related compounds as a basis of their therapeutic applications. Pages 1-28 in Ergot Alkaloids and Related Compounds. B. Berde and H.O. Schild, ed. Springer-Verlag, Berlin, Heidelberg, New York.

Berde, B. 1980. Ergot compounds: A synopsis. Pages 4-23 in Ergot Compounds and Brain Function: Neuroendocrine and Neuropsychiatric Aspects. M. Goldstein, A. Lieberman, D. B. Calne, and M. O. Thorner, ed. Raven Press, New York, NY.

Bernard, J. K., A. B. Chestnut, B. H. Erickson, and F. M. Kelly. 1993. Effects of prepartum consumption of endophyte-infected tall fescue on serum prolactin and subsequent milk production. J. Dairy Sci. 76:1928–1933.

Boling, J. A., L. P. Bush, R. C. Buckner, L. C. Pendlum, P. B. Burns, S. G. Yates, S. P. Rogovin, and H. L. Tookey. 1975. Nutrient digestibility and metabolism in lambs fed added perloline. J. Anim. Sci. 40:972-976.

Boling, J. A., L. D. Bunting, G. M. Davenport, J. L. Van der Veen, K. M. Meekins, N. W. Bradley, and R. E. Kohls. 1989. Physiological responses of cattle consuming tall fescue to environmental temperature and supplemental phenothiazine. J. Anim. Sci. 67:2377-2385.

Bolt, D. J. J. Bond, G. P. Lynch, and T. Elsasser. 1982. Concentrations of PRL, LH, FSH, GH, and TSH in plasma and pituitary of ewes grazing tall fescue and orchardgrass pastures. J. Anim. Sci. 55 (Suppl. 1):4 (Abstr.).

Bolt, D. J., J. Bond, and T. Elsasser. 1983. Changes in plasma prolactin and grazing behavior in heifers treated with dopamine antagonist while grazing tall fescue or orchardgrass pasture. J. Anim. Sci. 57 (Suppl. 1):48 (Abstr.).

Bond, J., J. B. Powell, and B. T. Weinland. 1984. Behavior of steers grazing several varieties of tall fescue during summer conditions. Agron. J. 76:707-709.

Bouton, J. H., R. N. Gates, D. P. Belesky, and M. Owsley. 1993a. Yield and persistence of tall fescue in the southeastern coastal plain after removal of its endophyte. Agron. J. 85:52-55.

Bouton, J. H., R. N. Gates, G. M. Hill, M. Owsley, and D. T. Wood. 1993b. Registration of 'Georgia 5' tall fescue. Crop Sci. 33:1405.

Bouton, J. H., R. R. Duncan, R. N. Gates, C. S. Hoveland, and D. T. Wood. 1997. Registration of 'Jesup' tall fescue. Crop Sci. 37:1011-1012.

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

Bransby, D. I., S. P. Schmidt, W. Griffey, and J. T. Eason. 1988. Heavy grazing is best for infected fescue. Alabama Agric. Exp. Sta. Highlights Agric. Res. 35(4):12.

Brown, M. A., L. M. Tharel, A. H. Brown, Jr., J. R. Miesner, and W. G. Jackson. 1992. Reproductive performance of Angus and Brahman cows grazing common bermudagrass or endophyte-infected tall fescue. Prof. Anim. Sci. 8:58-65.

Brown, M. A., L. M. Tharel, A. H. Brown, Jr., W. G. Jackson, and J. R. Miesner. 1993. Milk production in Brahman and Angus cows on endophyte-infected fescue and common bermudagrass. J. Anim. Sci. 71:1117-1122.

Brown, M. A., A. H. Brown, Jr., W. G. Jackson, and J. R. Miesner. 1996. Milk production in Angus, Brahman, and reciprocal-cross cows grazing common bermudagrass or endophyte-infected tall fescue. J. Anim. Sci. 74:2058-2066.

Brown, K. R., G. A. Anderson, K. Son, G. Rentfrow, L. P. Bush, J. L. Klotz, J. R. Strickland, J. A. Boling, and J. C. Matthews. 2009. Growing steers grazing high versus low endophyte (Neotyphodium coenophialum)-infected tall fescue have reduced serum enzymes, increased hepatic glucogenic enzymes, and reduced liver and carcass mass. J. Anim. Sci. 87:748-760.

Bruce, L. A. and T. L. Huber. 1973. Inhibitory effect of acid in the intestine on rumen motility in sheep. J. Anim. Sci. 37:164-168.

Bruce, K. D. W. Mega, K. Overheim, R. Cirz, R. Cass, M. Valderas, and R. Sherwood. 2013. Telemetry used to determine efficacy of intravenous Plazomicin against inhaled tularemia in Cynomolgus Macaques (CM). Abstract #120. Poster presented by Lovelace at the 13th Annual Meeting of the Safety Pharmacology Society, Rotterdam, Netherlands.

Buckner, R. C., L. P. Bush, and P. B. Burrus II. 1973. Variability and heritability of perloline in *Festuca* sp. *Lolium*, sp., and *Lolium-Festuca* hybrids. Crop Sci. 13:666-669.

Bultman, T. L. and N. J. Conard. 1998. Effects of endophytic fungus, nutrient level and plant damage on performance of fall armyworm (Lepidoptera: Noctuidae). Environ. Entomol. 27:631-635.

Bush, L. P., J. A. Boling, and S. G. Yates. 1979. Animal disorders. In: R. C. Buckner and L. P. Bush (eds.) Tall fescue. p. 247-292. Am. Soc. Agron., Madison, WI.

Bush, L. P., P. L. Cornelius, R. C, Buckner, D. R. Varney, R. A. Chapman, P. B. Burriss II, C. W. Kennedy, T. A. Jones, and M. J. Saunders. 1982. Association of N-acetyl loline and N-formyl loline with *Epichloe typhina* in tall fescue. Crop Sci. 22:941-943.

Bush, L. P. and P. B. Burrus, Jr. 1988. Tall fescue forage quality and agronomic performance as affected by the endophyte. J. Prod. Agric. 1:55-60.

Bush, L. P., H. H. Wilkinson, and C. L. Schardl. 1997. Bioprotective alkaloids of grass-fungal endophyte symbioses. Plant Physiol. 114:1-7.

Bush, L. P. and F. F. N. Fannin. 2009. Alkaloids. *In* H. A. Fribourg, D. B. Hannaway, and C. P. West (ed.) Tall fescue for the twenty-first century online monograph. Oregon State University Extension Service and University of Tennessee Agriculture Experiment Station. Agron. Monogr. 53. ASA, CSSA, and SSSA. Madison, WI.

Carlson, M. D., P. E. Kish, and T. Ueda. 1989. Glutamate uptake in synaptic vesicles: Competitive inhibition by bromocriptine. J. Neurochem. 53:1889-1894.

Chestnut, A. B., H. A. Fribourg, J. B. McLaren, D. G. Keltner, B. B. Reddick, R. J. Carlisle, and M. C. Smith. 1991. Effects of *Acremonium coenophialum* infestation, bermudagrass and nitrogen or clover on steers grazing tall fescue pastures. J. Prod. Agric. 4:208-212.

Chou, C. C. Intestinal blood flow regulation. In R. Dulbecco (Ed.), *Encyclopedia of Human Biology*. San Diego: Academic Press, 1992. Vol. 4. Pp. 547-556.

Church, D. C. 1976. *Digestive Physiology and Nutrition of Ruminants*. Vol. 1. Digestive Physiology. 2nd ed. Print. Published by D.C. Church, printed in Portland, Oregon.

Choudhary, M. S., N. Sachs, A. Uluer, R. A. Glennon, R. B. Westkaemper, and B. L. Roth. 1995. Differential ergoline and ergopeptine binding to 5-hydroxytryptamine$_{2A}$ receptors: Ergolines require an aromatic residue at position 340 for high affinity binding. Mol. Pharmacol. 47:450-457.

Christopherson, R. J. and P. M. Kennedy. 1983. Effect of the thermal environement on digestion in ruminants. Can. J. Anim. Sci. 63:477-496.

Clay, K., T. N. Hardy, and A. M. Hammond. 1985. Fungal endophytes of grasses and their effects on an insect herbivore. Oecologia 66:1-5.

Clay, K. and C. Schardl. 2002. Evolutionary origins and ecological consequences of endophyte symbiosis with grasses. Am. Nat. 160:S99-S127.

Cole, N. A., J. A. Stuedemann, C.W. Purdy, D. P. Hutchenson. 1987. Influence of endophyte in tall fescue pastures on the feedlot performance of feeder steers. J. Anim. Sci. 65 (Suppl. 1): 331 (Abstr.).

Colvin, H. W., Jr. and L. B. Daniels. 1965. Rumen motility as influences by physical form of oat hay. J. Dairy Sci. 48:935-941.

Cook, W. O., J. L. Richard, G. D. Osweiler, and D. W. Trampel. 1986. Clinical and pathological changes in acute bovine aflatoxicosis: Rumen motility and tissue and fluid concentrations of aflatoxins B1 and M1. Am. J. Vet. Res. 47 (8):1817-1825.

Cowan, J. R. 1956. Tall fescue. Adv. Agron. 8:283-320.

Crawford, Jr., R. J., J. R. Forwood, R. L. Belyea, and G. B. Garner. 1989a. Relationship between level of endophyte infection and cattle gains on tall fescue. J. Prod. Agric. 2:147-151.

Crawford, R. J., C. N. Cornell, M. Stokes, and G. Garner. 1989b. Management for restoring weight gain losses on infected pastures. In: Proc. Fescue Toxicosis Conference. pp 27-29. College of Agriculture, University of Missouri, Columbia.

Cunningham, I. J. 1949. A note on the cause of tall fescue lameness in cattle. Aust. Vet. J. 25:27-28.

Dado, R. G. and M. S. Allen. 1993. Continuous computer acquisition of feed and water intakes, chewing, reticular motility, and ruminal pH of cattle. J. Dairy Sci. 76:1589-1600.

Dahlman, D. L., M. R. Siegel, and L. P. Bush. 1997. Insecticidal activity of N-formyl loline. XVIII International Grasslands Congress. June 8-19, Winnipeg, Canada (in press).

Daniel, R. C. W. 1983. Motility of the rumen and abomasum during hypocalcemia. Can. J. Comp. Med. 47: 276-280.

De Lorme, M., S. Lodge-Ivey, and A. Craig. 2007. Physiological and digestive effects of *Neotyphodium coenophialum*-infected tall fescue fed to lambs. J. Anim. Sci. 85:1199-1206.

Duckett, S. K., J. G. Andrae, and S. L. Pratt. 2014. Exposure to ergot alkaloids during gestation reduces fetal growth in sheep. Frontiers in Chem. DOI: 10.3389/fchem.2014.00068.

Duncan, D. L. 1953. The effects of vagotomy and splanchnotomy on gastric motility in the sheep. J. Physiol. 119:157-169.

Dyer, D. C. 1993. Evidence that ergovaline acts on serotonin receptors. Life Sci. 53:PL223-PL228.

Dziuk, H. E. 1964. Radiotelemetry for stomach motility studies in ruminants. MedElectron. Biol. Engng. 2:281-287.

Dziuk, H. E. and E. H. McCauley. 1965. Comparison of ruminaoreticular motility patterns in cattle, sheep, and goats. Am. J. Physiol. 209:324-328.

Egert, A. M., D. H. Kim, F. N. Schrick, D. L. Harmon, and J. L. Klotz. 2014a. Dietary exposure to ergot alkaloids decreases contractility of bovine mesenteric vasculature. J. Anim. Sci. 92:1768-1799.

Egert, A. M., J. L. Klotz, K. R. McLeod, and D. L. Harmon. 2014b. Development of a methodology to measure the effect of ergot alkaloids on forestomach motility using real-time wireless telemetry. Front. Chem. DOI: 10.3389/fchem.2014.00090.

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

Ellis, J. L., R. E. Morrow, G. B. Garner, J. A. Stricker, and M. R. Ellersieck. 1983. Supplemental feeding of spring-calving cows on tall fescue or tall fescue-red clover pastures. J. Anim. Sci. 57:535-541.

Elmi, A. A., C. P. West, and Turner, K. F. 1989. Endophyte effects on leaf osmotic adjustment in tall fescue. In: Agronomy Abstracts. Am. Soc. Agron., Madison, WI, pg. 111.

Elmi, A. A., C. P. West, R. T. Robbins, and T. L. Kirkpatrick. 2000. Endophyte effects on reproduction of a root-knot nematode (*Meloidogyne marylandi*) and osmotic adjustment in tall fescue. Grass and Forage Sci. 55:166-172.

Fergus, E. N. 1952. Kentucky 31 fescue – Culture and use. Kentucky Agric. Ext. Cir. 497.

Fergus, E. N. and R. C. Buckner. 1972. Registration of Kentucky 31 tall fescue (Reg. No. 7). Crop Sci. 12:714.

Floss, H. G., J. M. Cassady, and J. E. Robbers. 1973. Influence of ergot alkaloids on pituitary prolactin and prolactin-dependent processes. J. Pharm. Sci. 62:699-715.

Foote, A. P., D. L. Harmon, J. R. Strickland, L. P. Bush, and J. L. Klotz. 2011. Effect of ergot alkaloids on contractility of bovine right ruminal artery and vein. J. Anim. Sci. 89:2944-2949.

Foote, A. P., D. L. Harmon, K. R. Brown, J. R. Strickland, K. R. McLeod, L. P. Bush, and J. L. Klotz. 2012. Constriction of bovine vasculature caused by endophyte-infected tall fescue seed extract is similar to pure ergovaline. J. Anim. Sci. 90:1603-1609.

Foote, A. P., N. B. Kristensen, J. L. Klotz, D. H. Kim, A. F. Koontz, K. R. McLeod, L. P. Bush, F. N. Schrick, and D. L. Harmon. 2013. Ergot alkaloids from endophyte-infected tall fescue decrease reticuloruminal blood flow and volatile fatty acid absorption from the washed reticulorumen. J. Anim. Sci. DOI: 10.2527/jas.2013-6517.

Foote, A. P. 2013. “Effect of ergot alkaloids on bovine foregut vasculature, nutrient absorption, and epithelial barrier function”. Theses and Dissertations – Animal and Food Sciences. Paper 18. http://uknowledge.uky.edu/animalsci_etds/18.

Forcherio, J. C., M. S. Kerley, and J. A. Paterson. 1992. Performance of steers grazing tall fescue during the spring followed by warm-season grasses during the summer. J. Anim. Sci. 70 (Suppl. 1):182 (Abstr.).

Forcherio, J. C., J. A. Paterson, and M.S. Kerley. 1993. Effects of supplemental energy or protein on rumen parameters and performance of first and second partum beef cows grazing tall fescue. J. Anim. Sci. 71 (Suppl. 1):197 (Abstr.).

Fribourg, H. A., A. B. Chestnut, R. W. Thompson, J. B. McLaren, R. J. Carlisle, K. D. Qwinn, M. C. Dixon, and M. C. Smith. 1991. Steer performance in fescue-clover pastures with different levels of endophyte infestation. Agron. J. 83:777-781.

Froetschel, M. A., W. J. Croom Jr., W. M. Hagler Jr., R. Argenzio, J. Liacos, and H. P. Broquist. 1986. Effects of slaframine on ruminant digestive function: Ruminal motility in sheep and cattle. J. Anim. Sci. 63:1502-1508.

Garman, H. 1900. Kentucky forage plants – The grasses. Kentucky Agric. Exp. Stn. Bull 87.

Garner, G. B., and C. N. Cornell. 1978. Fescue foot in cattle. In Mycotoxic Fungi, Mycotoxins, and Mycotoxicoses. T. D. Wylie and L. G. Morehouse, ed. 2:45-62. Marcel Dekker, New York, NY.

Gay, N., J. A. Boling, R. Dew, and D. E. Miksch. 1988. Effects of endophyte-infected tall fescue on beef cow-calf performance. Appl. Agric. Res. 3:182-186.

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

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

Goldstein, M., A. Lieberman, J.Y. Lew, T. Asano, M.R. Rosenfeld, and M.H. Makman. 1980. Interaction of pergolide with central dopaminergic receptors. Proc. Natl. Acad. Sci. USA 77:3725-3728.

Goddard, W. A., and R. Abrol. 2007. 3-Dimensional structures of G protein-coupled receptors and binding sites of agonists and antagonists. J. Nutr. 137:1528S-1538S.

Gregory, P. C. 1982. Forestomach motility in the chronically vagotomized sheep. J. Physiol. 328:431-447

Gregory, P. C. 1984a. Control of intrinsic reticulo-ruminal motility in vagotomized sheep. J. Physiol. 346:379-393.

Gregory, P. C. 1984b. Inhibition of forestomach motility by short-chain volatile fatty acids in sheep. Can. J. Anim. Sci. 64 (Suppl.): 11-12.

Grovum, W. L. 1986. Section I, part 2. The Control of Motility of the Ruminoreticulum. In: *Control of Digestion and Metabolism in Ruminants.* Proc. of the Sixth International Symposium on Ruminant Physiology. Sept. 10-14, 1984. Banff, Canada.

Gunter, S. A. and P. A. Beck. 2004. Novel endophyte-infected tall fescue for growing beef cattle. J. Anim. Sci. 82:E75-E82.

Harding, R. and B. F. Leek. 1972. Rapidly adapting mechanoreceptors in the reticulo-rumen which also respond to chemicals. J. Physiol. 223:32-33P.

Hardy, T. N., K. Clay, and A. M. Hammond, Jr. 1986. Leaf age and related factors affecting endphyte-mediated resistance to fall armyworm (Lepidoptera: Noctuidae) in tall fescue. Environ. Entomol. 15:1083-1089.

Hemken, R. W., J. A. Boling, L. S. Bull, R. H. Hatton, R. C. Buckner, and L. P. Bush. 1981. Interaction of environmental temperature and anti-quality factors on the severity of summer fescue toxicosis. J. Anim. Sci. 52:710-714.

Hemken, R. W., J. A. Jackson, Jr., and J. A. Boling. 1984. Toxic Factors in Tall Fescue. J. Anim. Sci. 58:1011-1016.

Henson, J. F. 2001. Plant Guide: Tall Fescue. USDA Natural Resources Conservation Service National Plant Data Center. <http://plants.usda.gov/plantguide/pdf/pg_loar10.pdf>.

Hill, N. S., D. P. Belesky, and W. C. Stringer. 1991. Competitiveness of tall fescue as influenced by *Acremonium coenophialum*. Crop. Sci. 31:185-190.

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

Hoveland, C. S., R. R. Harris, E. E. Thomas, E. M. Clark, J. A. McGuire, J. T. Eason, and M. E. Ruf. 1981. Tall fescue with ladino clover or birdsfoot trefoil as pasture for steers in northern Alabama. Auburn Univ. (Ala) Agric. Exp. Stn. Bull. 530.

Hoveland, C. S., S. P. Schmidt, C. C. King, Jr., J. W. Odom, E. M. Clark, J. A. Mcguire, L. A. Smith, H. W. Grimes, and J. L. Holliman. 1983. Steer performance and association of *Acremonium coenophialum* fungal endophyte on tall fescue pasture. Agron. J. 75:821-824.

Hoveland, C. S. 1993. Importance and economic significance of the *Acremonium* endophytes to performance of animals and grass plant. Agric. Ecosystems. Environ. 44:3-12.

Hoveland, C. S. 2009. Origin and History. *In* H. A. Fribourg, D. B. Hannaway, and C. P. West (ed.) Tall fescue for the twenty-first century online monograph. Oregon State University Extension Service and University of Tennessee Agriculture Experiment Station. Agron. Monogr. 53. ASA, CSSA, and SSSA. Madison, WI.

Hurley, W. L., E. M. Convey, K. Leung, L. A. Edgerton, and E. R. Hemken. 1980. Bovine prolactin, TSH, T4 and T3 concentrations as affected by tall fescue summer toxicosis and temperature. J. Anim. Sci. 51:374-379.

Jackson, Jr., J. A., R. W. Hemken, J. A. Boling, R. J. Harmon, R. C. Buckner, and L. P. Bush. 1984. Loline alkaloids in tall fescue hay and seed and their relationship to summer fescue toxicosis in cattle. J. Dairy Sci. 67:104-109.

Jensen, R., A. W. Deem, and D. Knaus. 1956. Fescue lameness in cattle. I. Experimental production of the disease. Am. J. Vet. Res. 17:196-201.

Johnson, M. C., D. L. Dahlman, M. R. Siegel, L. P. Bush, G. C. M. Latch, D. A. Potter, and D. R. Varney. 1985. Insect feeding deterrents in endophyte-infected tall fescue. Appl. Environ. Microbiol. 49:568-571.

Jones, T. A., R. C. Buckner, P. B. Burrus II, and L. P. Bush. 1983. Accumulation of pyrrolizidine alkaloids in benomy-treated tall fescue parents and their untreated progenies. Crop Sci. 23:1135-1140.

Julien, W. E., F. A. Martz, M. Williams, and G. B. Garner. 1974. Feed intake in Hereford calves infused intraperitoneally with toxic fescue extract. J. Dairy Sci. 57:1385-1387.

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

Kim, D. H., J. L. Klotz, and D. L. Harmon. 2013. Effect of endophyte-infected tall fescue seed on ruminal metabolism and physiology in Angus steers. J. Anim. Sci. 91: (E Suppl. 2) 522.

Klotz, J. L., L. P. Bush, D. L. Smith, W. D. Shafer, L. L. Smith, A. C. Vevoda, A. M. Craig, B. C. Arrington, and J. R. Strickland. 2006. Assessment of vasoconstrictive potential of D-lysergic acid using an isolated bovine lateral saphenous vein bioassay. J. Anim. Sci. 84:3167-3175.

Klotz, J. L., L. P. Bush, D. L. Smith, W. D. Schafer, L. L. Smith, B. C. Arrington, and J. R. Strickland. 2007. Ergovaline-induced vasoconstriction in an isolated bovine lateral saphenous vein bioassay. J. Anim. Sci. 85:2330-2336.

Klotz, J. L., B. H. Kirch, G. E. Aiken, L. P. Bush, and J. R. Strickland. 2008. Effects of selected combinations of tall fescue alkaloids on the vasoconstrictive capacity of fescue-naïve bovine lateral saphenous veins. J. Anim. Sci. 86:1021-1028.

Klotz, J. L., B. H. Kirch, G. E. Aiken, L. P. Bush, and J. R. Strickland. 2009. Bioaccumulation of ergovaline in bovine lateral saphenous veins *in vitro*. J. Anim. Sci. 88:408-415.

Klotz, J. L., B. H. Kirch, G. E. Aiken, L. P. Bush, and J. R. Strickland. 2010. Contractile-response of fescue-naïve, bovine lateral saphenous veins to increasing concentrations of tall fescue alkaloids. J. Anim. Sci. 88:408-415.

Klotz, J. L., K. R. Brown, Y. Xue, J. C. Matthews, J. A. Boling, W. R. Burris, L. P. Bush, and J. R. Strickland. 2012. Alterations in serotonin receptor-induced contractility of bovine lateral saphenous vein in cattle grazing endophyte-infected tall fescue. J. Anim. Sci. 90:682-693.

Klotz, J. L., G. E. Aiken, J. M. Johnson, K. R. Brown, L. P. Bush, and J. R. Strickland. 2013. Antagonism of lateral saphenous vein serotonin receptors from steers grazing endophyte-free, wild-type, or novel endophyte-infected tall fescue. J. Anim. Sci. 91:4492-4500.

Koontz, A. F., L. P. Bush, J. L. Klotz, K. R. McLeod, F. N. Schrick, and D. L. Harmon. 2012. Evaluation of a ruminally dosed tall fescue seed extract as a model for fescue toxicosis in steers. J. Anim. Sci. 90:914-921.

Koontz, A. F., D. H. Kim, K. R. McLeod, J. L. Klotz, and D. L. Harmon. 2014. Effect of fescue toxicosis on whole body energy and nitrogen balance, in situ degradation and ruminal passage rates in Holstein steers. Anim, Prod. Sci. http://dx.doi.org/10.1071/AN14037

Karg, H. and D. Schams. 1974. Prolactin release in cattle. J. Reprod. Fertil. 39:463-472.

Lamberts, S. W. J. and R. M. Macleod. 1990. Regulation of prolactin secretion at the level of the lactotroph. Physiol. Rev. 70:279-318.

Larson, B. T., M. D. Samford, J. M. Camden, E. L. Piper, M. S. Kerley, J. A. Paterson, and J. T. Turner. 1995. Ergovaline binding and activation of D2 dopamine receptors in GH4ZR7 cells. J. Anim. Sci. 73:1396-1400.

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

Latch, G. C. M., and M. J. Christensen. 1982. Ryegrass endophyte, incidence, and control. N. Z. J. Agric. Res. 25:443-448.

Lavker, R., W. Chalupa, and P. Opliger. 1969. Histochemical observations on rumen mucosa structure and composition. J. Dairy Sci. 52:266-269.

Leuchtmann, A., C. W. Bacon, C. L. Schardl, J. F. White, Jr., and M. Tadych. 2014. Nomenclatural realignment of Neotyphodium species with genus Epichloë. Mycologia 106:202-215.

Lipham, L. B., F. N. Thompson, J. A. Stuedemann, and J. L. Sartin. 1989. Effects of metoclopramide on steers grazing endophyte-infected fescue. J. Anim. Sci. 67:1090-1097.

Looper, M. L., T. S. Edrington, A. S. Moubarak, T. R. Callaway, and C. F. Rosenkrans. 2008. Effects of the ergot alkaloids dihydroergotamine, ergonovine, and ergotamine on growth of *Escherichia coli* O157:H7 *in vitro*. Foodborne Pathog. Dis. 5:599-604.

Lusby, K. S., W. E. McMurphy, C. A. Strasia, S. C. Smith, and S. H. Muntz. 1990. Effects of fescue endophyte and interseeded clovers on subsequent finishing performance of steers. J. Prod. Agric. 3:103-105.

Lyons, P. C. and C. W. Bacon. 1984. Ergot alkaloids in tall fescue infected with *Sphacelia typhina*. Phytopathology. 74:792.

Lyons, P. C., R. D. Plattner, and C. W. Bacon. 1986. Occurrence of peptide and clavine ergot alkaloids in tall fescue grass. Science 232:487-489.

Lyons, P. C., J. J. Evans, and C. W. Bacon. 1990. Effects of the fungal endophyte *Acremonium coenophialum* on nitrogen accumulation and metabolism in tall fescue. Plant Physiol. 92:726-732.

Maag, D. D. and J. W. Tobiska. 1956. Fescue lameness in cattle. II. Ergot alkaloids in tall fescue grass. Am. J. Vet. Res. 17:202-204.

Marple, D. N., T. G. Osborn, S. P. Schmidt, C. H. Rahe, and J. L. Sartin. 1988. Hypothalmic catecholamines in calves consuming fungus-infected, fungus-free, or fungus-free tall fescue plus ergotamine tartrate. J. Anim. Sci. 66 (Suppl. 1): 373 (Abstr.).

Maurer, R. A. 1981. Transcriptional regulation of the prolactin gene by ergocryptine and cyclic AMP. Nature 294:94-97.

Matheson, P. J., M. A. Wilson, R. N. Garrison. 2000. Regulation of Intestinal Blood Flow. J. Surgical Research. 93:182-196.

Maurer, R. A. 1981. Transcriptional regulation of the prolactin gene by ergocryptine and cyclic AMP. Nature 294:94-97.

McCulley, R. L., L. P. Bush, A. E. Carlisle, H. Ji, and J. A. Nelson. 2014. Warming reduces tall fescue abundance but stimulates toxic alkaloid concentrations in transition zone pastures of the U.S. Frontiers in Chem. 2. doi: 10.3389/fchem.2014.00088

McDonald, W. T., J. B. McClaren, H. A. Friboug, D. G. Keltner, and D. O. Onks. 1988. Finishing calves raised on fescue pastures with different levels of endophytic fungus infestation. J. Anim. Sci. 66 (Suppl. 1):57 (Abstr.).

McLeay, L., and B. Smith. 2006. Effects of ergotamine and ergovaline on the electromyographic activity of smooth muscle of the reticulum and rumen of sheep. Am. J. Vet. Res. 67: 707-714.

McMahon, M., G. Ward, P. Milliken, M. Aylott, and B. Patel. 2011. Assessment of non-invasive telemetric blood pressure and electrocardiogram measurement in conscious dogs. Poster presented by GlaxoSmithKline at the Society of Toxicology meeting, Washinton, D. C.

McMurphy, W. E., K. S. Lusby, S. C. Smith, S. H. Muntz, and C. A. Strasia. 1990. Steer performance on tall fescue pastures. J. Prod. Agric. 3:100-102.

McSweeney, C.S., P. M. Kennedy, and A. John. 1989. Reticulo-ruminal motility in cattle (*Bos indicus*) and water buffaloes (*Bubalus bubalis*) fed a low quality roughage diet. Comp. Biochem. Physiol. 94A (4):635-638.

Mooney, G. A., H. D. Radcloff, and J. J. Cupal. 1971. Effects of fistulation on rumen motility. J. Anim. Sci. 33:261 (Abstr.).

Mulac, D. and H. Humpf. 2011. Cytotoxicity and accumulation of ergot alkaloids in human primary cells. Toxicology 282:112-121.

Neil, W. D. and S. P. Schmidt. 1985. Effects of feeding Kentucky 31 tall fescue seed infected with *Acremonium coenophialum* to laboratory rats. J. Anim. Sci. 61:603-611.

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

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

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

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

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

Pavao-Zuckerman, M. A., J. C. Waler, T. Ingle, and H. A. Fribourg. 1999. Methane emissions of beef cattle grazing tall fescue pastures at three levels of endophyte infestation. J. Environ. Qual. 28:1963-1969.

Pedersen, J. F. and Rodriguez-Kabana. 1984. Differences among nematode populations in tall fescue pastures in north, central, and south Alabama. Crop Sci. 24:819-821.

Pedersen, J. F., R. Rodriguez-Kabana, and R. A. Shelby. 1988. Ryegrass cultivars and endophyte in tall fescue affect nematodes in grass and succeeding soybean. Agron. J. 80:811-814.

Pertz, H., and E. Eich. 1999. Ergot alkaloids and their derivatives as ligands for serotoninergic, dopaminergic, and adrenergic receptors. Pages 411-440 in Ergot the Genus Claviceps V. Kren and L. Cvak, ed. Harwood Academic Publishers, Amsterdam, Netherlands.

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

Poole, D., R. Littler, B. Smith, and L. Mcleay. 2009. Effects and mechanisms of action of the ergopeptides ergotamine and ergovaline and the effects of peramine on reticulum motility of sheep. Am. J. Vet. Res. 70: 270-276.

Porter, J.K., C.W. Bacon and J.D. Robbins. 1979. Ergosine, ergosinine, and chanoclavine I from *Epichloe typhina.* J. Agric. Food Chem. 27:595-598.

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

Read, J. C. and B. J. Camp. 1986. The effect of the fungal endophyte *Acremonium coenophialum* in tall fescue on animal performance, toxicity, and stand maintenance. Agron. J. 78:848-850.

Realini, C. E., S. K. Duckett, N. S. Hill, C. S. Hoveland, B. G. Lyon, J. R. Sackmann, and M. H. Gillis. 2005. Effect of endophyte type on carcass traits, meat quality, and fatty acid composition of beef cattle grazing tall fescue. J. Anim. Sci. 83:430-439.

Rhodes, M. T., J. A. Paterson, M. S. Kerley, H. E. Garner, and M. H. Laughlin. 1991. Reduced blood flow to peripheral and core body tissues in sheep and cattle induced by endophyte-infected tall fescue. J. Anim. Sci. 69:2033-2043.

Ribeiro, do Valle M. A. M, and L. P. Bush. 1983. Selection for low alkaloid content. p. 35-36. *In* Plant Sci. Crop Husbandry Res. Rep. The Agric. Inst. Headq., Dublin, Ireland.

Richardson, M. D., N. S. Hill, and C. S. Hoveland. 199-0a. Rooting patterns of endophyte infected tall fescue grown under drought stress. p. 129. *In* Agronomy Abstracts. ASA, CSSA, SSSA, Madison, WI.

Richardson, M. D., C. W. Bacon, and C. S. Hoveland. 1990b. The effect of endophyte removal on gas exchange in tall fescue. In: S. Quisenberry and S. Joost (Editors), Proc. Int. Symp. *Acremonium*/Grass Interactions. Louisiana Agric. Exp. Stn., Baton Rouge, LA, pg. 189-193.

Reid, C. S. W. and J. B. Cornwall. 1959. Mechanical activity of the reticulo-rumen of cattle. Proc. N. Z. Soc. Animal Prod. 19:23.

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

Ruckebusch, Y., C. H. Tsiamitas, and L. Bueno. 1972. The intrinsic electrical activity of the ruminant stomach. Life Sci. 11:55-64.

Ruckebusch, Y. and T. Tomov. 1973. The sequential contractions of the rumen associated with eructation in sheep. J. Physiol. 235:447-458.

Sampson, K. 1933. The systemic infection of grasses with *Epichloe typhina* (Pers.) Tul. Trans. Br. Myco. Soc. 18:30-47.

Schalk. A. F. and R. S. Amadon. 1928. Physiology of the ruminant stomach (bovine). Study of the dynamic factor. N. Dakota Agr. Exp. Sta. Bul. 216.

Schardl, C. L., A. Leuchtmann, and M. J. Spiering. 2004. Symbiosis of grasses with seedborne fungal endophytes. Annu. Rev. Plant Biol. 55:315-340

Schillo, K.K., L.S. Leshin, J.A. Boling, and N. Gay. 1988. Effects of endophyte-infected fescue on concentrations of prolactin in blood sera and the anterior pituitary and concentrations of dopamine and dopamine metabolites in brains of steers. J. Anim. Sci. 66:713-718.

Schmidt, S. P., C.S. Hoveland, E. M. Clark, N. D. Davis, L. A. Smith, H. W. Grimes, and J. L. Holliman. 1982. Association of an endophytic fungus with fescue toxicity in steers fed Kentucky 31 tall fescue seed or hay. J. Anim. Sci. 55:1259-1263.

Schmidt, S. P. and T. G. Osborn. 1993. Effects of endophyte-infected tall fescue on animal performance. Agric. Ecosystems Environ. 44:233-262.

Schnitzius, J. M., N. S. Hill, C. S. Thompson, and A. M. Craig. 2001. Semiquantitative determination of ergot alkaloids in seed, straw, and digesta samples using competitive enzyme-linked immunosorbent assay. J. Vet. Diagn. Invest. 13:230-237.

Schöning, C., M. Flieger, and H. H. Pertz. 2001. Complex interaction of ergovaline with 5-HT_{2A}, 5-$HT_{1B/1D}$, and alpha1 receptors in isolated arteries of rat and guinea pig. J. Anim.Sci. 79:2202-2209.

Schultz, C. L., S. L. Lodge-Ivey, L. P. Bush, A. M. Craig, and J. R. Strickland. 2006. Effects of initial and subacute exposure to an endophyte-infected tall fescue seed diet on faecal and urine concentrations of ergovaline and lysergic acid in mature geldings. N. Z. Vet. J. 54:178-184.

Sellers, A. F. and C. E. Stevens. 1966. Motor functions of the ruminant forestomach. Physiol. Rev. 46:634-661.

Shelby, R. A. and V. C. Kelley. 1990. An immunoassay for ergot-amine and related alkaloids. J. Agric. Food Chem. 38:1130-1134.

Shelby, R. A. and V. C. Kelley. 1992. Detection of ergot alkaloids from *Claviceps* species in agricultural products by competitive ELISA using a monoclonal antibody. J. Agric. Food Chem. 40:1090-1092.

Sibley, D.R. and I. Creese. 1983. Interactions of ergot alkaloids with anterior pituitary D2 dopamine receptors. Mol. Pharmacol. 23:585-593.

Siegel, M. R. M. C. Johnson, D. R. Varney, W. C. Nesmith, R. C. Buckner, L. P. Bush, P. B. Burrus II, T. A. Jones, and J. A. Boling. 1984a. A fungal endophyte in tall fescue: incidence and dissemintation. Phytopathology 74:932-937.

Siegel, M. R., D. R. Varney, M. C. Johnson, W. C. Nesmith, R. C. Buckner, L. P. Bush, P. B. Burrus II, and J. R. Hardison. 1984b. A fungal endophyte of tall fescue: Evaluation of control methods. Pytopathology 74:937-941.

Siegel, M. R., G. C. M. Latch, L. P. Bush, F. F. Fannin, D. D. Rowan, B. A. Tapper, C. W. Bacon, and M. C. Johnson. 1990. Fungal endophyte-infected grasses: alkaloid accumulation and aphid response. J. Chem. Ecol. 16:3301-3315.

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

Sleper, D. A., H. F. Maryland, R. J. Crawford, Jr., G. E. Shewmaker, and M. D. Massie. 2002. Registration of HiMag tall fescue germplasm. Crop Sci. 42:318-319.

Solomons, R. N., J. W. Oliver, and R. D. Linnabary. 1989. Dorsal pedal vein of cattle: Reactivity to selected ergot alkaloids associated with *Acremonium coenophialum*-infected fescue grass. Am. J. Vet. Res. 50:235-238.

Smith, D., L. Smith, W. Shafer, J. Klotz, and J. Strickland. 2009. Development and validation of an LC-MS method for quantification of ergot alkaloids in later saphenous vein tissue. J. Agric. Food Chem. 57:7213-7220.

Stevens, C. E. and B. K. Stettler. 1966. Factors affecting the transport of volatile fatty acids across the rumen epithelium. Am. J. Physiol. 210:365-372.

Strahan, S. R., R. W. Hemken, J. A. Jackson, Jr., R. C. Buckner, L. P. Bush, and M. R. Siegel. 1987. Performance of lactating dairy cows fed tall fescue forage. J. Dairy Sci. 70:1288-1234.

Strickland, J. R., M. L. Looper, J. C. Matthews, C. F. Rosenkrans Jr., M. D. Flythe, and K. R. Brown. 2011. St. Anthony's Fire in livestock: Causes, mechanisms, and potential solutions. J. Anim. Sci. 89:1603-1626.

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

Tan, C. M., A. E. Brady, H. H. Nickols, Q. Wang, and L. E. Limbird. 2004. Membrane trafficking of G protein-coupled receptors. Annu. Rev. Pharmacol. Toxicol. 44:559-609.

Thompson, F. N., J. A. Stuedemann, J. L. Sartin, D. P. Belesky, and O. J. Devine. 1987. Selected hormonal changes with summer fescue toxicosis. J. Anim. Sci. 65:727-733.

Thompson, F. N. and J. A. Stuedemann. 1993. Pathophysiology of fescue toxicosis. Agric. Ecosystems Environ. 44:263-281.

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

Tucker, C. A., R. E. Morrow, J. R. Gerrish, C. J. Nelson, G. B. Garner, V. E. Jacobs, W. G. Hires, J. J. Shinkle, and J. R. Forwood. 1989. Forage systems for beef cattle: Calf and backgrounded steer performance. J. Prod. Agric. 2:208-213.

Turner, K. E., J. A. Paterson, M. S. Kerley, and J. R. Forwood. 1990. Mefluidide treatment of tall fescue pastures: Intake and animal performance. J. Anim. Sci. 68:3399-3405.

Van Soest, P. J. 1994. *Nutritional ecology of the ruminant*. 2nd ed. Print. Cornell University Press. Ithaca, New York.

Vinall, H. N. 1909. Meadow fescue; its culture and uses. USDA Farmers Bull. 361.

Waghorn, G. C. and C. S. W. Reid. 1983. Rumen motility in sheep and cattle given different diets. N. Z. J. Agric. Res. 26:289-295.

Waller, J. C., J. B. McLaren, H. A. Fribourg, A. B. Chestnut, and D. G. Keltner. 1989. Effect of *Acremonium coenophialum* infected *Festuca arundinacae* on cow-calf performance. In: Proc. 16th Int. Grassl. Congr., Oct. 4-11, Nice, France. p. 1193.

Waller, J. C. 2009. Endophyte Effects on Cattle. *In* H. A. Fribourg, D. B. Hannaway, and C. P. West (ed.) Tall fescue for the twenty-first century online monograph. Oregon State University Extension Service and University of Tennessee Agriculture Experiment Station. Agron. Monogr. 53. ASA, CSSA, and SSSA. Madison, WI.

Washburn, S. P., J. T. Green, Jr., and B. H. Johnson. 1989. Effects of endophyte presence in tall fescue on growth, puberty, and conception in Angus heifers. In: Proc. Tall Fescue Toxicoses Workshop. November 13-14. p. 80. Southern Region Information Exchange Group 37 (SRIEG-37). Atlanta, GA.

Weber, H. P. 1980. The molecular architecture of ergopeptines: A basis for biological interaction. Pages 25-34 in Ergot Compounds and Brain Function: Neuroendocrine and Neuropsychiatric Aspects. M. Goldstein, A. Lieberman, D. B. Calne, and M. O. Thorner, ed. Raven Press, New York, NY.

West, C. P., E. Izekor, D. M. Oosterhuis, and R. T. Robbins. 1988. The effect of *Acremonium coenophialum* on the growth and nematode infestation of tall fescue. Plant Soil 112:3-6.

West, C. P., E. Izekor, A. Elmi, R. T. Robbins, and K. E. Turner. 1989. Endophyte effects on drought tolerance, nematode infestation and persistence of tall fescue. In: C. P. West (Editor), Proceedings of the Arkansas Fescue Toxicosis Conference, 10 July 1989, Boone, AK, No. 140. Arkansas Agric. Exp. Stn., University of Arkansas, Fayetteville, AK, pg. 23-27.

West, C. P., E. Izekor, K. E. Turner, and A. A. Elmi. 1993. Endophyte effects on growth and persistence of tall fescue along a water-supply gradient. Agron. J. 85:264-270.

Westendorf, M. L., G. E. Mitchell, and R. E. Tucker. 1992. Influence of rumen fermentation on response to endophyte-infected tall fescue seed measured by a rat bioassay. Drug Chem. Toxicol. 15:351-364.

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

Wilkinson, S. R., J. A. Stuedemann, and D. J. Williams. 1983. Animal performance on tall fescue: Fat necrosis. Proc. Tall Fescue Toxicosis Workshop, Atlanta, GA. Univ. of Georgia Ext., Athens, GA.

Williams, M., S. R. Shaffer, G. B. Garner, S. G. Yates, H. L. Tookey, L. D. Kintller, S. L. Nelson, and J. T. McGinity. 1975. Induction of fescue foot syndrome in cattle by fractionated extracts of toxic fescue hay. Am. J. Vet. Res. 36:1353-1357.

Wyburn, R. S. 1980. Section I, part 1: The mixing and propulsion of the stomach contents in ruminants. In: *Digestive Physiology and Metabolism in Ruminants*. Proceedings of the 5th International Symposium on Ruminant Physiology. Ferrand, Sept. 3-7, 1979.

Xue, Y., S. F. Liao, J. R. Strickland, J. A. Boling, and J. C. Matthews. 2011. Bovine neuronal vesicular glutamate transporter activity is inhibited by ergovaline and other ergopeptines. J. Dairy Sci. 94:3331-3341.

Yates, S. G. 1962. Toxicity of tall fescue forage: A review. Econ. Bot. 16:295-303.

Yates, S. G., R. D. Plattner, and G. B. Garner. 1985. Detection of ergopeptine alkaloids in endophyte infected, toxic Ky-31 tall fescue by mass spectrometry/mass spectrometry. J. Agric. Food Chem. 33:719.

Yates, S. G. and R. G. Powell. 1988. Analysis of ergopeptine alkaloids in endophyte-infected tall fescue. J. Agric. Food Chem. 36:337-340.

VITA

Amanda M. Egert
Birthplace: Saginaw, Michigan

Education:

2012 – 2014 (expected)	*University of Kentucky – Lexington, KY* Degree: Master of Science in Animal Science Emphasis: Ruminant Nutrition Advisor: David L. Harmon, Ph.D. Thesis: Effect of dietary exposure to ergot alkaloids on contractility of bovine mesenteric vasculature and rumen motility.
2008 – 2012	*Michigan State University – East Lansing, MI* Degree: Bachelor of Science in Animal Science Emphasis: Animal Biology Graduation with High Honors

Professional Experience:

2012 – Present	*Graduate Research Assistant* Department of Animal and Food Sciences University of Kentucky – Lexington, KY
2011	*Merritt Entomology Lab Assistant* Michigan State University – East Lansing, MI

Teaching Experience:

2013-2014	*Laboratory Methods in Nutritional Sciences Teaching Assistant* University of Kentucky – Lexington, KY
2011	*Animal Feeding and Nutrition Lab Teaching Assistant* Michigan State University – East Lansing, MI

Awards and Honors:

2014	*Outstanding Graduate Student Award – M.S. Division* Gamma Sigma Delta University of Kentucky – Lexington, KY
2012	*Board of Trustees' Scholarship Award* Michigan State University – East Lansing, MI

Publications:

Peer-reviewed Publications

Egert, A. M., J. L. Klotz, K. R. McLeod, and D. L. Harmon. 2014. Development of a methodology to measure the effect of ergot alkaloids on forestomach motility using real-time wireless telemetry. Front. Chem. DOI: 10.3389/fchem.2014.00090.

Egert, A. M., D. H. Kim, F. N. Schrick, D. L. Harmon, and J. L. Klotz. 2014. Dietary exposure to ergot alkaloids decreases contractility of bovine mesenteric vasculature. J. Anim. Sci. 92 (4): 1768-1779.

Abstracts

Egert, A. M., K. R. McLeod, J. L. Klotz, and D. L. Harmon. 2014. Analysis of rumen motility patterns using a wireless telemetry system to characterize bovine reticuloruminal contractions. J. Anim. Sci. (E-Suppl. 2): 1641.

Egert, A. M., D. H. Kim, D. L. Harmon, and J. L. Klotz. 2013. Dietary exposure to ergot alkaloids decreases contractility of bovine mesenteric vasculature. J. Anim. Sci. 91 (E-Suppl. 2): 214.

Copyright © Amanda M. Egert 2014