TIMING OF EXPOSURE TO TOXIC TALL FESCUE: EFFECTS ON BEEF CATTLE REPRODUCTION

Matthew Burns
Clemson University, burns5@clemson.edu

Follow this and additional works at: https://tigerprints.clemson.edu/all_dissertations
Part of the Animal Sciences Commons

Recommended Citation
Burns, Matthew, "TIMING OF EXPOSURE TO TOXIC TALL FESCUE: EFFECTS ON BEEF CATTLE REPRODUCTION" (2012). All Dissertations. 927.
https://tigerprints.clemson.edu/all_dissertations/927

This Dissertation is brought to you for free and open access by the Dissertations at TigerPrints. It has been accepted for inclusion in All Dissertations by an authorized administrator of TigerPrints. For more information, please contact kokeefe@clemson.edu.
TIMING OF EXPOSURE TO TOXIC TALL FESCUE: EFFECTS ON BEEF CATTLE REPRODUCTION

A Dissertation
Presented to
the Graduate School of
Clemson University

In Partial Fulfillment
of the Requirements for the Degree
Doctor of Philosophy
Animal and Veterinary Sciences

by
Matthew Gary Burns
May 2012

Accepted by:
Dr. John G. Andrae, Committee Chair
Dr. Susan K. Duckett
Dr. Scott L. Pratt
Dr. F. Neal Schrick
ABSTRACT

Tall fescue [Schedonorus phoenix (Scop.) Holub] is a native grass of Europe and was not widely used in the U.S. until the early to mid 1900’s. Toxic tall fescue (E+) is the predominate, cool-season forage used in beef cattle production in the southern U.S., but grazing this forage is known to negatively impact growth and may impair reproductive performance. Two- and 3-yr old (young) beef cattle respond differently to synchronization protocols and have higher energy requirements compared with mature cows under normal management conditions. Interestingly, limited data indicate that young cattle reproductive performance is negatively impacted to a greater extent than mature cattle when grazing E+. Toxic tall fescue effects on pregnancy rate have been documented, but specific timing of exposure and negative events remain unclear. Even though the specific mechanisms associated with decreased reproductive performance are uncertain, alternative grazing systems have been developed to attenuate female reproductive problems and alleviate decreased calf growth performance associated with E+ fescue grazing. The objectives of these studies were to determine if grazing E+ negatively impacts reproduction pre- or post-insemination, and to evaluate calf performance associated with exposure to toxic and non-toxic forage. Preliminary data from a local producer’s cattle operation indicated a reduction in timed artificial insemination (TAI) pregnancy rates due to E+ exposure. In addition, 2- and 3-yr old (young) cattle tended ($P = 0.07$) to have reduced TAI pregnancy rates compared with $\geq$ 4-yr old (mature) cattle. In a follow-up study, young cattle grazing E+ pre-TAI had lowered ($P < 0.05$) d 30, 60 and 130 pregnancy rates when compared with grazing non-
toxic forages (O). Grazing E+ post-AI lowered d 60 and 130 pregnancy rates compared to O treatment ($P < 0.05$). Pre-weaning calf growth was negatively affected by E+ treatment post-AI as average daily gain (ADG) and 205-d adjusted weaning weight (ADJ WW) were both decreased ($P < 0.05$) in calves grazing E+ post-AI compared with calves grazing O post-AI. Grazing 2- and 3-yr old beef cattle on toxic tall fescue prior to the breeding season decreases timed artificial insemination (TAI) and natural service pregnancy rates. Specific aims have been designed to systematically define the point at which E+ negatively impacts beef cattle reproduction and performance.
DEDICATION

I would like to dedicate this work to my loving wife, Dr. Ashley Burns. Without her help the three letters “PhD” would not have ever been behind my name. I would also like to dedicate it to my parents Gary and Sarah Burns, and sister Cameron Burns. I really appreciate all your help and prayers. Without your support and help, this would not have been possible.
ACKNOWLEDGEMENTS

Thank you Dr. Andrae for making this possible. I have thoroughly enjoyed my time spent working on these projects. You always have an open door policy and an ear ready to listen. I am grateful for the research experience as well as your help in molding me into a successful extension educator.

Thank you to Dr. Duckett, Dr. Pratt, and Dr. Schrick for advice and guidance on various physiological aspects related to my project. Thank you for being patient and challenging me to think outside my comfort zone.

I would like to thank everyone who helped me with my project, especially Gary Burns. My father was instrumental in the execution of these projects and always willing to listen to theories and frustration while sitting on the tailgate of the truck. I really appreciate all of his help and support. Maggie Miller was my personal “heat watch” consultant and always willing to help work cows or do lab work, thanks Maggie. Special thanks to all the undergraduate students, graduate students, and beef farm staff for help with animal work. I really appreciate Dr. Payton in Schrick Lab for help with progesterone and prolactin assays. Thanks to Dr. Billy Bidges for help with statistical analysis of my data. Special thanks to Extension Field Operations, Heather Repec, Dr. Callahan, and Dr. Meadows for support and guidance. Special thanks to Joe Davis and family for all of their work and support.

A big “Thank You” to Dr. Ashley Burns, my wife, for all of her support and help. She is an excellent record keeper/tuber labeler. Without her support during the writing phase, this would not have been possible. I love you and thank you very much.
TABLE OF CONTENTS

Page

TITLE PAGE .................................................................................................................... i
ABSTRACT ..................................................................................................................... ii
DEDICATION ................................................................................................................ iv
ACKNOWLEDGEMENTS ............................................................................................. v
LIST OF TABLES ......................................................................................................... vii
LIST OF FIGURES ...................................................................................................... viii
CHAPTER

1. FESCUE TOXICOSIS IN BEEF CATTLE PRODUCTION
   Introduction .............................................................................................. 1
   Tall Fescue ............................................................................................... 2
   Fescue Toxicosis ...................................................................................... 4
   Alleviation of Fescue Toxicosis............................................................. 11
   Literature Cited ...................................................................................... 15

2. TALL FESCUE NEGATIVELY AFFECTS REPRODUCTIVE SUCCESS IN
   BEEF CATTLE
   Abstract .................................................................................................. 19
   Introduction ............................................................................................ 21
   Materials and Methods ........................................................................... 23
   Results and Discussion .......................................................................... 24
   Literature Cited ...................................................................................... 30

3. TIMING OF EXPOSURE TO TOXIC TALL FESCUE NEGATIVELY
   AFFECTS REPRODUCTIVE SUCCESS IN TWO- AND THREE-YEAR OLD
   BEEF COWS
   Abstract .................................................................................................. 31
   Introduction ............................................................................................ 33
   Materials and Methods ........................................................................... 35
   Results and Discussion .......................................................................... 38
   Literature Cited ...................................................................................... 49

4. INVESTIGATING MECHANISM OF FESCUE TOXICOSIS IN BEEF
   CATTLE
   Project Summary .................................................................................... 52
   Project Narrative .................................................................................... 53
   Literature Cited ...................................................................................... 69
### LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1. Cumulative pregnancy rates for cows that grazed toxic tall fescue (E+) or other non-toxic forages (O; bermudagrass and annual ryegrass) pre- and post-AI</td>
<td>47</td>
</tr>
<tr>
<td>3.2. Independent pregnancy rates for cows that grazed toxic tall fescue (E+) or other non-toxic forages (O; bermudagrass and annual ryegrass) pre- and post-AI</td>
<td>48</td>
</tr>
</tbody>
</table>
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1. Structural similarities between ergot alkaloids and neurotransmitters</td>
<td>14</td>
</tr>
<tr>
<td>2.1. Research timeline and fixed-time artificial insemination (TAI) estrous synchronization protocol</td>
<td>26</td>
</tr>
<tr>
<td>2.2. Pregnancy rates of 2- and 3-yr old (young) and mature cows grazing wild-type endophyte-infected fescue (E+) 14 d prior to initiation of synchronization protocol</td>
<td>27</td>
</tr>
<tr>
<td>2.3. Natural service pregnancy rates for all beef cows (A) and mature cows (B) grazing wild-type endophyte-infected fescue (E+) or novel endophyte-infected (NE+) beginning 14 d prior to initiation of the breeding season</td>
<td>28</td>
</tr>
<tr>
<td>2.4. Final pregnancy rates for all beef cows (A) and 2- and 3-yr old (young) and mature cows (B) wild-type endophyte-infected fescue (E+) or novel endophyte-infected (NE+) beginning 14 d prior to initiation of the breeding season</td>
<td>29</td>
</tr>
<tr>
<td>3.1. Serum prolactin concentrations of cattle grazing endophyte-infected (E+) or non-toxic forages (O) pre-artificial insemination (AI) and post-AI (year 1, d 10; year 2, d 30) relative to timed AI (d 0)</td>
<td>43</td>
</tr>
<tr>
<td>3.2. Cumulative pregnancy rates following timed AI (1st pregnancy diagnosis on d 30) and natural service (2nd and final pregnancy check d 60 and 130 respectively) for cows grazing toxic fescue (E+) pre- and post-AI (E+E+), E+ pre-AI and non-toxic forages (O) post-AI (E+O), O grasses pre-AI and E+ post AI (OE+), and O grasses (OO) pre- and post-AI</td>
<td>44</td>
</tr>
<tr>
<td>3.3. Adjusted 205-d weaning weight for calves grazing toxic tall fescue (E+) or non-toxic (O) forages in the pre- and post-AI periods</td>
<td>45</td>
</tr>
<tr>
<td>3.4. Average daily gain (ADG) for calves grazing toxic tall fescue (E+) or non-toxic (O) forages in the pre- or post-AI periods</td>
<td>46</td>
</tr>
</tbody>
</table>
List Of Figures (Continued)

Figure

4.1. Serum prolactin concentrations of cattle grazing endophyte-infected (E+) or non-toxic forages (O) pre-artificial insemination (AI) and post-AI (year 1, d 10; year 2, d 30) relative to timed AI (d 0) .............................................57

4.2. Cumulative pregnancy rates following timed AI (1\textsuperscript{st} pregnancy diagnosis on d 30) and natural service (2\textsuperscript{nd} and final pregnancy check d 60 and 130 respectively) for cows grazing toxic fescue (E+) pre- and post-AI (E+E+), E+ pre-AI and non-toxic forages (O) post-AI (E+O), O grasses pre-AI and E+ post AI (OE+), and O grasses (OO) pre- and post-AI ..................58
CHAPTER 1: FESCUE TOXICOSIS IN BEEF CATTLE PRODUCTION

INTRODUCTION

Tall fescue [Schedonorus phoenix (Scop.) Holub] is the predominant, cool-season perennial forage in much of the southern United States with 8.5 million cattle grazing wild-type, endophyte-infected fescue (Hoveland, 1993). Tall fescue is a native grass of Europe and was not widely used in the U.S. until the early to mid 1900’s. Dr. E. N. Fergus discovered and acquired seed to later release the Kentucky 31 (KY-31) variety of tall fescue (Hoveland, BIF symposium paper need to add to citation), which is an ideal forage due to length of growing season, pest resistance, adaptability, and ease of establishment. The discovery of the KY-31 fescue variety, which remains green throughout winter, revolutionized grazing and livestock production in the Southeast. Most varieties of tall fescue grown in the U.S. achieve superior performance through a symbiotic relationship with an endophytic fungus. Neotyphodium coenophialum, the endophyte present in KY-31 fescue, produces ergot alkaloids that are beneficial to the plant, but can be detrimental to livestock when consumed (Hill et al., 1994). Fescue toxicosis is a syndrome that develops in livestock grazing tall fescue and is characterized by decreased blood flow to extremities, increased core body temperature, and poor growth and reproductive performance (Porter, 1995). The loss of value attributed to decreased production traits totals over $600 million annually (Allen and E. Segarra, 2001), adjusted for inflation is approaching $1 billion today.
TALL FESCUE

Tall Fescue [*Schedonorus phoenix* (Scop.) Holub] is a deep-rooted, cool-season perennial bunchgrass. It is used widely in the U.S. for turf and forage applications with over 100 varieties adapted to different situations. Tall fescue has spring and fall growing seasons and can also be stockpiled for winter grazing. Extending the grazing season transitions production away from stored forage and concentrates for winter-feeding and, therefore, decreases costs associated with livestock production. Fescue could be considered an ideal forage due to its long growing seasons, pest resistance, adaptability, ease of establishment, and grazing and drought tolerance. Wild-type infected tall fescue is widely used for soil conservation, reclamation, turf, and pasture establishment. From the Mid-Atlantic region to the Southeast, approximately 16 million hectares are planted in fescue. Widespread use of this forage is attributed to tall fescue’s highly adaptive nature due to a mutualistic symbiosis with *N. coenophialum* (Panaccione et al., 2001). *Neotyphodium coenophialum* is the most widely studied endophyte in the U.S. due to its relationship with tall fescue. It produces compounds, such as ergot alkaloids, which provide the grass with resistance to environmental stressors. Alkaloids are responsible for insect resistance and drought tolerance for the plant (Clay and Schardl, 2002). Also, loline alkaloids provided by the endophyte give fescue more insect resistance (Riedell et al., 1991).

Ergot alkaloids (Figure 1) are comprised of an ergoline ring, which makes them very similar in structure to the neurotransmitters: epinephrine, dopamine, norepinephrine, and serotonin (Berde, 1980; Larson, 1995). Ergovaline, the primary
member of a larger class of compounds known as ergopeptines, is the most prominent
ergot alkaloid as it accounts for 84 to 97% of the total fraction (Yates et al., 1985; Lyons
et al., 1986). Each ergopeptine consists of a lysergic acid ring and 3 amino acids. The
variation in amino acid structure distinguishes the different ergopeptine compounds.
Ergovaline consists of a lysergic acid ring and L-alanine, L-valine, L-proline amino acids
(Panaccione et al., 2001) and it is a known dopamine agonist (Jones et al., 2003). Ergot
alkaloids are similar in structure to dopamine and have been shown to bind to the D2
dopamine receptor with the same affinity as dopamine (Berde and Sturmer, 1978; Larson
et al., 1995). Research suggests the dopaminergic pathway explains the decreased levels
of circulating serum prolactin (PRL) associated with fescue toxicosis (Schillo et al., 1988;
Porter and Thompson, 1992). Prolactin has been implicated in over 300 known
biological functions, and has been shown to be luteotrophic in rodents stimulating the
production of progesterone (P4) to maintain pregnancy. However, PRL’s role in
ruminant physiology is less clear as it does not appear to be luteotrophic. Therefore, the
role of prolactin in bovine reproduction is not well understood and is not known to affect
reproductive performance when depressed in fescue toxicosis.
FESCUE TOXICOSIS

Despite tall fescue’s many positive attributes as a grazing forage, its association with *N. coenophialum*, and subsequent production of ergot alkaloids, it can be detrimental to livestock production. Consumption of the endophyte and associated compounds by livestock induces a syndrome known as fescue toxicosis. Fescue toxicosis is characterized by decreased blood flow to extremities, increased core body temperature, and poor growth and reproductive performance (Porter, 1995). Animal toxicity problems due to forage can be traced back as far as 50 A.D. (Matthew 13:25-40) and can affect cattle, sheep, and horses. Fungus infected seed of darnel was associated with toxicity problems in both humans and animals (Bacon, 1995). Although fungus toxicity problems have been researched for hundreds of years, we still have a limited knowledge of exact mechanisms and causative agents leading to decreased animal performance and negative associative effects.

Because ergovaline is the most predominant ergot alkaloid produced by fescue’s endophyte, its ingestion by livestock is thought to cause toxicosis (Yates et al., 1985; Lyons et al., 1986). Hill et al. (2001) alternatively suggest that lysergic acid, specifically, may be the causative agent of fescue toxicosis. Lysergic acid and lysergol have greater transport potential through digestive tract tissue in vitro when compared with ergopeptine alkaloids. However, research continues to focus on ergopeptine compounds as the causative agent(s) of toxicosis symptoms in livestock.
Temperature Regulation and Vasoconstriction. Environmental conditions exacerbate toxic effects; vasoconstriction causes problems with heat dissipation in hyperthermic conditions, and problems with loss of extremities in extreme hypothermic conditions. In addition, cattle grazing tall fescue often exhibit dull, rough hair coats and are slow to shed winter hair in spring. Heat stress in hyperthermic environments can change grazing behavior and depress animal intake. Decreased blood flow in cattle can cause heat stress in hyperthermic ambient conditions and loss of extremities (fescue foot) in hypothermic ambient conditions (Strickland et al., 1993). Aiken et al. (2007) demonstrated vasoconstriction of the caudal artery 4 h after feeding a toxic-endophyte diet. Decreased blood flow to the lungs and possible effects on red blood cell function decreased oxygenation of circulating blood. In order to compensate for decreased oxygenation of the blood, respiration rate is often elevated in cattle grazing toxic tall fescue. It is unclear which alkaloids are responsible for vasoconstrictive activity in the bovine. In a study by Klotz et al. (2008), biopsied bovine lateral saphenous veins were taken from fescue-naïve, crossbred heifers to examine their contractile response to different ergot alkaloids. Ergovaline was found to have the highest contractile response when compared to lysergic acid and N-acetylloloine. However, there was an additive effect when ergovaline and lysergic acid were combined, leading to the conclusion that multiple alkaloid compounds contribute to vasoconstriction in vivo. Current research suggests vasoconstriction of smooth muscle may continue for extended periods after removal from toxic exposure (Pratt et al., - unpublished).

Animal Growth and Carcass Traits. Decreased weight gains associated with stocker cattle grazing toxic tall fescue is well documented (Stuedemann and Hoveland,
Meta-analysis of several, individual studies and pooled, grazing studies of multiple locations and years has revealed an inversely proportional, linear correlation between endophyte infection level and average daily gain (ADG).

There is a 45 g/d decrease in ADG for every 10% increase in endophyte incidence in a fescue stand (Thompson et al., 1993). Decrease in weight gain may be attributed to lower dry matter intake (DMI) and, therefore, a lower plane of nutrition for cattle grazing toxic fescue. In addition, it has been postulated that decreased DMI may be attributed to decreased foregut movement due to alkaloid binding of smooth muscle receptors.

Decreases in live weight, ADG, and hot carcass weight (HCW) have all been documented with only minor effects on carcass quality in pasture-based systems (Realini et al., 2005). Realini et al. (2005) also documented ergot alkaloid presence in adipose tissue of steers grazing toxic tall fescue. Storage and release of ergot alkaloids from adipose tissue may offer an explanation for delayed toxicosis symptoms well after toxic grazing exposure.

Reproduction. Beef cattle reproductive efficiency can lead to success or failure of an operation. Unfortunately in the Southeast, the majority of cow-calf operations have spring breeding seasons when endophyte levels in fescue and ambient temperatures are the highest. The combination of high endophyte levels and heat stress exacerbate the effects of fescue toxicosis. Consumption of wild-type infected tall fescue has been shown to decrease reproductive performance (Porter and Thompson, 1992; Brown et al., 2000; Looper et al., 2009; Looper et al., 2010). There is also conflicting evidence that suggests fescue does not have an effect on conception rate or pregnancy loss (Burke and Rorie, 2002). Variations in results of reproductive studies can partly be attributed to variations in environmental conditions such as season, ambient temperature and
humidity. Environmental impact on fescue toxicosis has been well documented. Exposure to wild-type infected tall fescue under heat stress conditions magnifies any deleterious effects on reproduction and weight gain. Therefore, if subacute levels of toxic fescue are consumed in an isothermal environment, there may be no effect of grazing toxic tall fescue on cattle reproduction.

In addition to environmental effects, some variations in the reported literature may be attributed to epigenetic effects. Calf performance due to dam intrauterine environment or subsequent milk production may also be possible sites of epigenetic effects. More research is needed to determine possible heritable traits or adaptations that allow cattle to improve performance while exposed to toxic tall fescue. Studies have shown that Zebu-influenced cross-breeding programs may offset some of the deleterious effects associated with fescue toxicosis (Brown et al., 2000). It is believed that any benefit attributed to the Zebu-influenced is gained from increased heat tolerance. Although some studies have examined breed differences, it is not known if certain bloodlines within breeds can tolerate fescue better than others. Future studies should focus on the long-term effects of extended exposure to toxic tall fescue from generation to generation.

Effect on the Bovine Male. Effects of E+ on male bovine fertility have not been well documented. Two studies were designed to determine fescue toxicosis effects on male fertility by exposing bulls to toxic tall fescue grazing or administering ergotamine tartrate (Schuenemann et al., 2005a; Schuenemann et al., 2005b). Ergotamine tartrate is used to simulate exposure to toxic tall fescue. Each study performed standard semen evaluation every 60 d, and retained a portion of the semen for in vitro fertilization. While
no motility or morphological differences were detected in either study, embryos fertilized with sperm exposed to the toxic treatment exhibited lower cleavage rates when compared with controls (Schuenemann et al., 2005a; Schuenemann et al., 2005b). Therefore, sperm produced under toxic fescue conditions may have decreased capacity to fertilize oocytes, resulting in a reduction of cleaved embryos. Semen morphology and motility was decreased in Brahman-influenced bulls grazing toxic tall fescue relative to bulls grazing novel endophyte-infected fescue. These effects were amplified with increased maximum ambient temperatures (Looper et al., 2009).

**Ovarian Function.** Research shows toxic tall fescue in combination with heat stress has direct effects on ovarian function. In a study by Burke et al. (2001), heifers were fed endophyte-free (E-) or endophyte-infected (E+) seed and exposed to either thermoneutral or heat stress conditions. The size of corpus luteum (CL) did not differ between treatment groups (Burke et al., 2001). However, circulating serum progesterone (P4) concentrations were decreased in heifers exposed to both E+ and heat stress conditions. Number and size of large follicles were also decreased in heifers exposed to both E+ and heat stress conditions compared with all other treatment groups (Burke et al., 2001). In 2003, Jones et al. conducted a study investigating P4 concentrations in cattle grazing E-, E+ and E+ with domperidone administration. The authors reported lower circulating P4 relative in cattle grazing E+ compared with E- and E+ with domperidone administration, but no treatment differences in P4 production from luteal cells (Jones et al., 2003). Differences in circulating P4 could be attributed to decreased blood flow to the corpus luteum, or decreased total circulating cholesterol, which is a precursor to P4 production.
It has also been reported that heifers exposed to endophyte-infected seed exhibited shorter estrous cycles, and decreased serum P4 concentrations during mid-cycle (Jones et al., 2003). In a microarray performed by Jones et al. (2004), gene expression of luteal tissue was altered between cattle exposed to endophyte-infected seed compared with non-infected seed. Cattle fed endophyte-infected seed had 598 genes up-regulated and 56 genes down regulated compared with cattle fed non-infected seed. The D2 dopamine receptor was not reported, however, G protein coupled receptor 9, which is similar to the D2 dopamine receptor, was down-regulated at the 3-fold level. As previously stated, the D2 receptor is responsible for the down regulation of PRL in response to exposure to toxic tall fescue. Although serum P4 and gene expression levels are altered, direct impact of ability to conceive and maintain pregnancy is not apparent. Ergot alkaloids decreased circulating concentration of luteinizing hormone (LH) and increased PGF$_{2\alpha}$ metabolite in lactating Holstein cows (Browning et al., 1998). Luteinizing hormone is instrumental in the development and maintenance of a functional CL, and PGF$_{2\alpha}$ instrumental in the demise of a functional CL. Although the CL does not respond to PGF$_{2\alpha}$ prior to d 7 after estrus, this may offer some explanation for early embryonic loss in cattle grazing toxic tall fescue.

*Embryo Development.* It is unclear whether negative impacts on reproduction occur pre- or post-fertilization. Embryo development and early embryonic death have been the focus of research efforts to determine the point(s) of interest in fescue exposure. In a study by Schuenemann et al. (2005c), embryo quality was assessed between cattle receiving no treatment and cattle receiving ergotamine tartrate. More embryos collected from control animals developed to stage 4 embryos (88%) compared to ergotamine
tartrate treated animals (56%). Cleavage rates were not affected (Schuenemann et al., 2005c). In male fertility studies, a decrease in cleavage rates from embryos fertilized by E+ exposed sperm has been documented (Schuenemann et al., 2005b). Therefore, male and female exposure to toxic fescue may contribute to decreased reproductive performance in different ways. Embryos collected from donor cattle not treated with ergotamine tartrate were implanted on d 7 post-estrus in control and ergotamine tartrate-treated recipients. There was no difference in pregnancy rates between each group (Schuenemann et al., 2005c). Therefore, female exposure to toxic tall fescue most likely impacts reproductive performance negatively during fertilization, embryo development, or both prior to d 7 post-ovulation.
ALLEVIATION OF FESCUE TOXICOSIS

Since diagnosis of fescue toxicosis in livestock, research efforts have targeted ways to decrease production loss while still utilizing tall fescue to maximum potential. In certain species, toxicosis symptoms can be ameliorated through the use of a dopamine agonist. Endophyte-free varieties of tall fescue were developed, but lacked ability to persist when compared to wild-type endophyte infected (Hill et al., 1991). Development of novel endophyte-type varieties of tall fescue have been successful in maintaining plant vigor without negative affects on livestock.

_Dopamine Agonist_. Research in other species would suggest that domperidone, a dopamine agonist, is successful in modulating certain effects caused by grazing toxic tall fescue. Heifers grazing E+ fescue exhibited shorter estrous cycles and lower mid-cycle serum P4 concentrations relative to heifers grazing E- fescue or E+ with domperidone treatment. Cattle in the E+ treatment had lower weight gains when compared to the E- and E+ with domperidone treatment groups (Jones et al., 2003). These results suggest that domperidone may ameliorate certain symptoms of fescue toxicosis. However, treatment of grazing cattle during the breeding season on a large scale does not seem to be logistically or economically viable.

_Novel Endophytes_. Presently, research focuses have targeted development of “novel” endophytes, which provide positive symbiotic relationships for the plant while not producing ergot alkaloids responsible for fescue toxicosis (Bouton et al., 2002). When grazed by sheep and stocker cattle, the novel endophyte-infected varieties (NE+) had similar average daily gains when compared to E- (Parish et al., 2003a; Parish et al.,
With 8.5 million acres of E+ fescue being grazed, it is obvious that producers are not going to eliminate all toxic tall fescue and establish new varieties. Therefore, developments of strategies to utilize this adaptable forage with the least impact on livestock production have been beneficial.

Stockpiled Tall Fescue. It has been shown that amount of standing forage does not change in stockpiled fescue from mid-December through March. Ergovaline concentrations, however, decreased during that same time (Kallenbach et al., 2003). In addition, thermal stress during the winter months is less of a factor for grazing E+ fescue. The combinations of lowered ergovaline concentrations in stockpiled E+ fescue and decreased ambient temperatures may reduce animal production losses due to fescue toxicosis.

Dilution Effects. Interseeding clover into existing wild-type E+ fescue stands can “dilute” toxicity. Thompson et al. (1993) combined grazing studies to show that ADG was not affected despite adding clover in stands of fescue with high infection levels compared to stands of fescue with low infection levels and no clover. Therefore, most improvement can be seen in fescue stands with low infections levels. Average daily gain was increased in the low to moderately infected stands by including clover. The inconsistency between studies can be largely attributed to the drastic difference in environmental conditions across the regions where fescue is grown. Including other grasses, clovers, or both in existing fescue stands is largely dependent on environmental conditions during establishment, and increased animal performance would be impacted. Inclusion of clovers in fescue stands not only dilutes the toxic effects, but also improves the quality of forage in the pasture, which could also explain the increase in animal gains.
Rotational Grazing. Rotational grazing is becoming a more widely adopted management practice for general forage management of tall fescue. Research has focused on the benefits to forage production and utilization, and not necessarily production concerns with reference to toxicosis. Little to no differences have been detected with intermittent grazing of E+ fescue vs. E- or NE+ fescue. Equine research suggests that horses must be removed from toxic tall fescue 30 to 90 d prior to foaling to avoid any deleterious effects to mare or foal at parturition. Timing of grazing rotation and breeding season could have significant impacts on reproductive efficiency in beef cattle. Based on low ergot alkaloid concentrations and low ambient temperatures, a late fall breeding season would be ideal to improve reproduction efficiency. Further research is needed in this area to develop a systematic approach for rotational grazing of E+ and non-toxic forages by the same animals within a given year.
Figure 1. Structural similarities between ergot alkaloids and neurotransmitters. Ergovaline (A) contains lysergic acid (B), which includes ergoline (C). The ergoline ring is very similar in structure to several neurotransmitters, including serotonin (D) and epinephrine (E).


Hoveland, C. S. 1993. Economic importance of \textit{Acremonium} endophytes. Agriculture Ecosystems and Environment. 44(3)


CHAPTER 2: TALL FESCUE NEGATIVELY AFFECTS REPRODUCTIVE SUCCESS IN BEEF CATTLE.

ABSTRACT

Toxic tall fescue [Schedonorus phoenix (Scop.) Holub] is the predominate, cool-season forage used in beef cattle production in the southern U.S., but grazing this forage negatively impacts animal growth and reproductive performance. Data was collected and reported from a local beef cattle producer operation in Oconee County, South Carolina. Fourteen days prior to the initiation of synchronization protocol, Brangus x Angus cows, stratified by breed and age, were allocated to graze wildtype endophyte-infected tall fescue (E+; n = 50) or novel endophyte-infected fescue (NE+; n = 31) throughout the breeding season (60 d). Cattle grazing E+ were subjected to a standard CO-Synch + controlled internal drug release (CIDR) estrous synchronization protocol followed 64 h later by a timed artificial insemination (TAI); NE+ cattle did not undergo the synchronization protocol or TAI. Natural Service sires were turned out with E+ and NE+ cattle 10 d post-TAI of the E+ group. No NE+ TAI control was conducted and total pregnancy rates were determined by rectal palpation at d 100 post-TAI. Younger females may have more difficulty when grazing E+ as 2- and 3-yr old cattle grazing E+ tended (P = 0.07) to have lower TAI pregnancy rate than mature cows grazing E+, 35 ± 7.5% and 13.3 ± 9.1% respectively. Cattle grazing E+ had lower (P < 0.05) natural service (E+, 59.6 ± 7.2%; NE+, 90.5 ± 7.7%) and final pregnancy rates (E+ 68.3 ± 5.6%; NE+ 90.5 ± 7.6%) than cattle grazing NE+. Although not significant, 2- and 3-yr old (young) cattle grazing E+ experienced numerically lower (P > 0.05) natural service (young 46.2 ± 8.3%);
mature 69.2 ± 11.8%) and final pregnancy rates (young 56.6 ± 7.6%; mature 80.0 ± 9.3%) than mature cattle grazing E+. Therefore, moving cows to E+ 14 d prior to the breeding season negatively impacts reproductive performance of beef cows. In addition, the effect may be more detrimental on 2- and 3-yr old cattle compared with mature cows.
INTRODUCTION

Reproduction is the limiting factor of profitability and production efficiency of a beef cattle operation (Dickerson, 1970; Dziuk and Bellows, 1983). Nutritional management and body condition of primiparous cattle significantly impacts reproductive success (Ciccioli et al., 2003). Beef cattle operations in the southern United States rely on forages and grazing to meet nutritional needs of these animals. Tall Fescue [Schedonorus phoenix (Scop.) Holub] is the predominant cool-season perennial forage in much of the southern United States with 8.5 million cattle grazing 32 million acres (Hoveland, 1993). Utilization of fescue allows more grazing days during the fall/winter, and spring of the year when warms season forages are not producing. Wild-type endophyte-infected fescue (Hoveland, 1993) is associated with decreased reproductive performance in cattle (Schmidt et al., 1986; Gay et al., 1988; Clay and Sbardul, 2002); however, specific mechanisms of how or at what stage of the reproductive cycle E+ negatively impacts reproductive performance are unknown. Even with the uncertainty of how E+ decreases reproductive performance, alternative grazing systems have been proposed to attenuate female reproductive problems associated with fescue grazing. While cultivars of non-toxic tall fescue have been developed and are becoming more widely used, most forage systems in the southeastern U.S. are still based on E+. Since toxic fescue is such a large part of most cow-calf grazing systems, grazing management strategies need to be examined to determine if animal reproductive performance can be improved. However, knowledge of when toxic tall fescue grazing impacts reproductive performance would lead to better grazing strategies. The objective of this study was to
determine if cattle conditioned on non-toxic forage can be transitioned to toxic tall fescue immediately prior to the breeding season without decreasing reproductive performance.
MATERIALS AND METHODS

Data was collected and reported from a local beef cattle producer operation in Oconee County, South Carolina. Two-year old (n = 32), 3-yr old (n = 16), and mature (n = 33) Brangus x Angus cows were grazing novel endophyte-infected tall fescue (NE+). Fourteen days prior to initiation of synchronization protocol, cows were stratified by breed and age and allocated to graze E+ (n = 50) or NE+ (n = 31) throughout the breeding season.

Estrus and ovulation were synchronized using a CO-Synch + CIDR protocol in beef cattle grazing E+ (Figure 1). In brief, an controlled internal drug release (CIDR) was inserted and 100 µg of GnRH (Cysterilin) was administered (i.m.) on d -10. On d -3, the CIDR was removed and 25 mg of PGF$_{2\alpha}$ (Lutalyse) was injected (i.m.). Fixed-time artificial insemination (TAI) was performed on d 0, 60 to 64 h after PGF$_{2\alpha}$ injection. Pregnancy was diagnosed at 33 d post-TAI and pregnancy survival was verified 35 d later. Natural service sires were turned out with both NE+ and E+ cows 10 d after TAI for E+ group.

The statistical design of the data set was a two-factor treatment structure consisting of treatment (E+, NE+) with a completely randomized experiment structure. Chi-square tests based on a generalized linear model for the data set were used to determine significant differences among the treatment percentages for quantitative responses such as pregnancy rates. All tests were performed with $\alpha = 0.05$ and all calculations were performed using JMP (Version 9.0. SAS Institute Inc., Cary, NC).
RESULTS AND DISCUSSION

Artificial insemination pregnancy rates for cattle grazing E+ were numerically decreased compared with expected pregnancy rates using a CO-Synch+CIDR protocol in 2 and 3 yr old beef cattle (Figure 2). Although there was not a non-toxic grazing treatment control for TAI pregnancy rates in this study, an expected pregnancy rate of 60% was achieved by the same AI technician using a similar protocol, time of year, and genetic makeup of cattle for two years in a row (Peterson et al., 2011). Interestingly, 2 and 3 yr old cattle tended ($P = 0.07$) to have lower TAI pregnancy rates compared with mature cows. Therefore, rotating cattle from NE+ to E+ two weeks prior to start of the breeding season appears to negatively impact reproductive performance. However, the timing at which the negative effects occur is unknown. The negative effect of E+ grazing could be post-fertilization; however, it is possible that E+ negatively impacts the developmental competence of the oocyte ovulated during the CO-Sync procedure lowering fertilization rates. Future studies will be conducted to determine stages of the female reproductive cycle that is affected by E+ treatment.

Natural service (Figure 3A) and final pregnancy (Figure 4A) rates were decreased ($P < 0.05$) in cattle grazing E+ compared with cattle grazing NE+. Although not significant, 2- and 3-yr old beef cows grazing E+ had the numerically lower natural service (Figure 3B) and final (Figure 4B) pregnancy rates compared with 2- and 3-yr old cows grazing NE+ or mature cows grazing E+ or NE+. Decreased reproductive performance in response to grazing E+ has been well documented (Schmidt et al., 1986; Gay et al., 1988); however, effects specific to 2- and 3-yr old beef cattle along with
timing need to be addressed. Heifers are historically maintained as a separate group, but are managed with the mature cow herd after their initial calf. Two and 3 yr old beef cattle respond differently to reproductive management strategies (Burns et al., 2008). Therefore, these effects may be more prominent in production settings, but the performance of the 2- and 3-yr old animals has been masked by mature cow performance on E+.

Toxic tall fescue is the predominant cool season forages used by beef producers in the Southeast U.S. Beef producers have begun to implement alternative fescue varieties to assist in alleviating these negative impacts. It is imperative that future research focuses on the timing and duration of exposure to E+ which results in decreased production through decreased reproductive performance and growth potential. Management strategies will have to allow utilization of existing E+ forage, but must maximize beef cattle production and performance by minimizing exposure to E+ fescue during specific time points in the production system. These results are preliminary information for the research that will be presented in the next chapter.
Figure 1. Research timeline and fixed-time artificial insemination (TAI) estrous synchronization protocol. Cows were grazed on endophyte-infected fescue (E+) beginning 14 d prior to initiation of estrous synchronization protocol using gonadotropin releasing hormone (GnRH), internal progesterone releasing device (CIDR), and prostaglandin F$_{2\alpha}$ (PGF$_{2\alpha}$).
Figure 2. Pregnancy rates of 2- and 3-yr old (young) and mature cows grazing wild-type endophyte-infected fescue (E+) 14 d prior to initiation of synchronization protocol. (A) Pregnancy rates due to timed artificial insemination (TAI). Dotted line represents approximate industry average using same synchronization protocol. # Pregnancy rates tended to differ ($P = 0.07$) between young and mature beef cows. (B) Distribution of final pregnancy rates due to TAI and natural service. Age was not statistically significant ($P = 0.27$) nor was there an interaction between age and forage ($P = 0.62$), but 2- and 3-yr old cows grazing E+ had the numerically lowest final pregnancy rates due to lower TAI rates.
Figure 3. Natural service pregnancy rates for all beef cows (A) and 2- and 3-yr old (young) and mature cows (B) grazing wild-type endophyte-infected fescue (E+) or novel endophyte-infected fescue (NE+) beginning 14 d prior to initiation of the breeding season. *Main effect of forage on final pregnancy rate ($P < 0.05$). Age was not statistically significant ($P = 0.40$) nor was there an interaction between age and forage ($P = 0.78$), but 2- and 3-yr old cows grazing E+ had the numerically lowest natural service pregnancy rates.
Figure 4. Final pregnancy rates for all beef cows (A) and 2- and 3-yr old (young) and mature cows (B) grazing wild-type endophyte-infected fescue (E+) or novel endophyte-infected fescue (NE+) beginning 14 d prior to initiation of the breeding season. *Main effect of forage on final pregnancy rate ($P < 0.05$). Age was not statistically significant ($P = 0.27$) nor was there an interaction between age and forage ($P = 0.62$), but 2- and 3-yr old cows grazing E+ had the numerically lowest final pregnancy rates.
LITERATURE CITED


CHAPTER 3: TIMING OF EXPOSURE TO TOXIC TALL FESCUE NEGATIVELY AFFECTS REPRODUCTIVE SUCCESS IN TWO AND THREE YEAR OLD BEEF COWS

ABSTRACT

The impact of tall fescue infected with the toxic wild-type endophyte (E+) on beef cattle reproductive performance has received limited attention, and few studies examine the effect of exposure timing on conception rates. The objective of this study was to determine if grazing E+ negatively impacts reproduction pre- or post-insemination, and to evaluate calf performance associated with exposure to toxic and nontoxic forage. To determine if E+ altered reproduction during gamete development or by altering uterine environment, cattle were exposed to E+ prior to or immediately following insemination.

In a multi-year grazing study, 2- and 3-yr old beef cows (114 cows total) were blocked by breed, body condition score (BCS) and age and allotted to treatment groups grazing E+ (> 70% wild-type infected) or other nontoxic forages (O; common bermudagrass and annual ryegrass) for 90 d prior to timed artificial insemination (TAI). According to the estrous synchronization protocol, all animals received a CIDR 8 d prior to AI, which was removed after 5 d followed by 2 injections of PGF$_{2\alpha}$ 8 h apart. Timed AI was performed 72 ± 2 h post-CIDR removal. Immediately following TAI, approximately 50% of cows from each original grazing treatment were switched to the alternate grazing treatment for the remainder of the trial (130 d), consistent with a 2x2 factorial arrangement. The statistical design of the data set was a two-factor factorial treatment structure consisting of pre-AI treatment (E+, O) and post-AI treatment (E+, O) with a completely randomized
experiment structure. The experimental design resulted in the following treatment combinations: fescue-fescue (E+E+, n = 32), fescue-other (E+O, n = 26), other-fescue (OE+, n = 30), and other-other (OO, n = 36). Ten days after TAI, bulls were placed with cows for 60 d. Blood was collected on d -18 and -8 for progesterone (P4) analysis to assess cyclicity. Blood was also collected pre- (d -18) and post-AI (year 1 = d 10; year 2 = d 30) for prolactin (PRL) concentrations. Pregnancy was determined using transrectal ultrasonography at d 30, 60, and 130 and verified with calving records. Fescue exposure decreased serum PRL levels ($P < 0.05$) compared to O. Grazing E+ pre-AI lowered ($P < 0.05$) d 30, 60 and 130 pregnancy rates when compared to O. Grazing E+ post-AI lowered d 60 and 130 pregnancy rates compared to O treatment ($P < 0.05$). Pre-weaning calf growth was negatively affected by E+ treatment post-AI as ADG and adjusted weaning weight (ADJ WW) were both decreased ($P < 0.05$) in calves grazing E+ post-AI compared with calves grazing O post-AI. Grazing 2 and 3 yr old beef cattle on toxic tall fescue prior to the breeding season decreases TAI and final pregnancy rates.
INTRODUCTION

Reproduction is a major limiting factor of profitability and production efficiency of a beef cattle operation (Dickerson, 1970; Dziuk and Bellows, 1983). Nutritional management and body condition of primiparous cattle significantly impacts reproductive success (Ciccioli et al., 2003). Beef cattle operations in the Southern United States typically rely on grazed forages to meet nutritional needs of these animals. Tall fescue \textit{[Schedonorus phoenix (Scop.) Holub]} is the predominant cool-season perennial forage in much of the Southeast and Mid-Atlantic regions of the U.S. with 8.5 million cattle grazing 32 million acres of wild-type endophyte infected (E+) fescue (Hoveland, 1993). Decreased reproductive performance in cattle grazing E+ fescue has been documented (Schmidt et al., 1986; Gay et al., 1988; Clay and Schardl, 2002; Schuenemann et al., 2005a); however, specific mechanisms of how or at what stage of the reproductive cycle toxic tall fescue negatively impacts reproductive performance remains unknown. In addition, the literature is unclear as to whether E+ negatively impacts gamete development, fertilization, or early embryonic development. Decreased performance of calves grazing E+ is also well documented (Schmidt et al., 1982; Stuedemann et al., 1986; Watson et al., 2004). Even though the specific mechanisms associated with decreased reproductive performance are uncertain, alternative grazing systems have been developed to attenuate female reproductive problems and alleviate decreased calf growth associated with E+ fescue grazing. Addition of clover to existing toxic tall fescue stands was successful at alleviating reproductive effects in mature cows, but pregnancy rates remained decreased in 2 and 3 yr old beef cattle (Gay et al., 1988). While cultivars of non-toxic tall fescue are becoming more widely used, most forage systems in the
southeastern U.S. still utilize predominantly toxic tall fescue. However, if used the non-toxic or novel endophyte containing forages achieve similar performance as endophyte-free or other forages (Watson et al., 2004). Therefore, grazing management strategies using E+ based forage systems should be further examined to determine if animal reproductive performance can be improved. Specifically, knowledge of when E+ fescue grazing negatively impacts reproductive and calf performance may lead to grazing strategies that improve conception rates. The objective of this study was to determine if altering the timing of exposure of toxic tall fescue during the breeding season can improve reproductive performance of 2- and 3-yr old beef cows and calf performance.
MATERIALS AND METHODS

This experiment was conducted at the Simpson Beef Cattle Experiment Station, Dalton Farm located approximately 11 km southeast of Clemson, SC in accordance with approved Clemson University Animal Care and Use Committee protocol. Two (n = 64) and 3 (n = 50) year old beef cows and their calves were blocked by cow breed, body condition score (BCS), and age and allotted to groups grazing E+ (> 70% wild-type infected; n = 58) or other forages (O; common bermudagrass and annual ryegrass; n = 56) for 90 d prior to timed artificial insemination (TAI). Cow body condition score (BCS) was evaluated d -100 prior to onset of treatment and 10 d prior to TAI on d 0. Immediately following TAI, approximately half of cow-calf pairs from each group were switched to the alternate grazing treatment for the remainder of the trial stratified by cow breed, BCS, age, and days post-partum (PP). Consistent with a 2x2 factorial arrangement, the experimental design resulted in 4 total forage treatment combinations when grazed pre- and post-AI: fescue-fescue (E+E+, n = 32), fescue-other (E+O, n = 26), other-fescue (OE+, n = 30), and other-other (OO, n = 26).

On d -8, all cows were subjected to a standard, 5 d-CIDR estrous synchronization program followed by TAI. The 5d-CIDR protocol consisted of d -8 insertion of CIDR and injection of GnRH (i.m., 100 µg), d -3 removal of CIDR and injection of PGF2α (i.m., 25 mg) followed 8 h later with second injection of PGF2α (i.m., 25 mg), and d 0 TAI and injection of GnRH (i.m., 100 µg). Estrus behavior was visually determined after the second PGF2α injection until d 0. Cows exhibiting estrus behavior within 48 h of
second PGF$_{2\alpha}$ injection were inseminated 12 h post-estrus detection and all remaining cows were TAI on d 0. Ten days post-timed AI, bulls were placed with cows for 60 d.

**Prolactin and Progesterone.** Blood was collected via coccygeal venapuncture on d -18 and -8 for progesterone (P4) analysis to assess cyclicity. Blood was also collected on pre-AI (d -8) and post-AI (year 1 = d 10; year 2 = d 30) for PRL concentrations to assess toxicosis. Prolactin and P4 concentrations were analyzed using radioimmunoassay as described by Forrest et al., (1980) as modified by Bernard et al., (1993) and Progesterone Coat-A-Count Kit (Siemens Healthcare Solutions; Deerfield, IL), respectively. Assays were performed by Schrick Laboratory, University of Tennessee (Knoxville, TN).

**Reproductive Performance.** Pregnancy was determined using transrectal ultrasonography at d 30, 60, and 130 and verified with calving records. Pregnancy rates are reported in two ways: 1) cumulative pregnancy rates with d 30, 60, and 130 pregnancy rates being inclusive of the previous time points and 2) independent pregnancy rates with pregnancy rates at each time point being reported separately. Independent pregnancy rates at d 0 are reflective of conception rates due to TAI. Similarly, pregnancy rates from d 1 to 30 and d 30 to 60 are reflective of natural service pregnancy rates established in open cows within each time period.

**Calf Performance.** Calf weights were obtained at birth and weaning. Calves were weaned approximately 4 mo post-AI and average daily gains (ADG) and adjusted weaning weights were calculated to 205 d using the standard adjustments for cow age.

The statistical design of the data set was a two-factor factorial treatment structure consisting of pre-AI treatment (E+, O) and post-AI treatment (E+, O) with a completely

36
randomized experiment structure. A two-way analysis of variance (ANOVA) followed by Fisher’s t-tests based on a general linear model of the data set were used to determine significant differences among the treatment means for quantitative responses such as PRL, P4, calf ADG, and calf ADJ WW. Chi-square tests based on a general linear model for the data set were used to determine significance among the treatment percentages for quantitative responses such as pregnancy rates. All tests were performed with alpha = 0.05 and all calculations were performed using JMP (Version 9.0. SAS Institute Inc., Cary, NC).
RESULTS AND DISCUSSION

Prolactin and Progesterone. Cattle grazing E+ pre-AI (d -18) had lower ($P < 0.05$) serum PRL levels ($10.15 \pm 8.70$ ng/ml) than cattle grazing O ($79.86 \pm 8.83$ ng/ml). Cattle grazing E+ post-AI (year 1 = d 10; year 2 = d 30) had lower ($P < 0.05$) serum PRL levels ($34.09 \pm 10.86$ ng/ml) than cattle grazing O ($116.76 \pm 11.85$ ng/ml; Figure 1). There was no treatment by year interaction ($P > 0.05$) for PRL. Decreased PRL concentrations have been reported in mammalian species exposed to E+ fescue or ergot alkaloids and are often used as an indicator of fescue toxicosis (Hurley et al., 1981; Schillo et al., 1988b; Mizinga et al., 1993; Bernard et al., 1993). With a decrease of approximately 80% in PRL concentrations, our results are consistent with signs of fescue toxicosis (Elsasser and Bolt, 1987; Schillo et al., 1988a).

Progesterone data was used to determine cycling status prior to insertion of the CIDR device. Cycling status did not significantly ($P > 0.05$) impact pregnancy rate. Body condition scores and days PP at TAI were not different ($P > 0.05$) for pre or post AI trt.

Reproductive Performance. There was a main effect of pre-AI forage treatment on cumulative pregnancy rates. Grazing E+ pre-AI decreased cumulative pregnancy rates ($P < 0.05$) at d 30, 60, and 130 compared with O pre-AI. In addition, cattle grazing E+ post-AI had lower ($P \leq 0.01$) d 60 and 130 cumulative pregnancy rates compared with O post-AI (Table 1). There was no difference ($P > 0.05$) in late pregnancy loss (occurring after d 30) between cattle grazing E+ or O, consistent with (Burke et al., 2001; Waller et al., 2001). Schuenemanm et al. (2005b) showed no difference in pregnancy rate via
transcervical implantation of embryos on d 7 post-estrus between cattle recipients exposed to ergotamine tartrate and control treatments. Therefore, these results suggest impacts of E+ on pregnancy rates are occurring before implantation or maternal recognition of pregnancy. We propose that E+ impacts pregnancy rates prior to or immediately following insemination. Proper timing of ovulation is important for the success of TAI. Cattle exposed to E+ diets have shorter estrous cycles (Jones et al., 2003), which may require different timing of insemination, or have immature oocytes due to shorter estrous cycle duration or early ovulation. Alternatively, Seals et al. (2005) showed no change in follicular dynamics in beef heifers exposed to ergotamine tartrate as a model for fescue toxicosis, but pregnancy rates were still reduced. In this study, grazing E+ fescue decreases pregnancy rates in 2 and 3 yr old beef cows, which is consistent with previously reported studies (Gay et al., 1988; Porter and Thompson, 1992; Brown et al., 2000). Interestingly, Gay et al. (1988) utilized clover in E+ to improve pregnancy rate in mature cows, but 2- and 3-yr old cow pregnancy rate did not respond. Nutritional management and body condition significantly impacts reproduction in primiparous beef cattle (Ciccioli et al., 2003). Two- and 3-yr old beef cattle are still actively growing to mature body weight, and therefore have increased energy demands compared to mature cows in the same stage of production. This may offer partial explanation for the differences in reproductive performance when exposed to E+.

Although there was no interaction ($P = 0.46$) of pre-AI and post-AI grazing treatment, cows switched from O to E+ following insemination had numerically lower cumulative pregnancy rates (83.2 and 96.8 % for OE+ and OO, respectively) compared with OO; cows switched from E+ to O had numerically increased cumulative pregnancy
rates (93.1 and 60.4 % for E+O and E+E+, respectively) compared with E+E+ (Figure 2). Therefore, decreased cumulative pregnancy rates seen in E+ grazing may be slightly alleviated by switching to non-toxic forages. Caldwell et al. (2010) showed that grazing cattle on non-toxic forage 28 d prior to and partially during the breeding season would achieve similar calving rates as control treatment. This system still allows utilization of existing E+ forage while utilizing non-toxic forages to manage and offset poor reproductive performance. Pregnancy rates examined in ranges (Table 2) indicate decreased ($P < 0.05$) d 1 - 30 natural service pregnancy rates in response to post-AI E+ exposure. Although this is a post-AI effect according to the study design, the impacts of E+ on cow reproduction may still be attributed to grazing E+ prior to natural service breeding. Because pregnancy rates established by TAI were not affected by post-AI grazing treatment, data suggests that E+ does not impact the maintenance of pregnancy once it is established. The effect of E+ on reproductive performance may be at the level of oocyte maturation, ovulation, fertilization, or early embryo development prior to d 30.

In addition, low natural service rates achieved from cattle grazing E+ during breeding season may also be attributed, in part, to decreased bull fertility, as bulls were also grazing E+ forage. Decreased reproductive performance has been reported in bulls exposed to ergot alkaloids (Schuenemann et al., 2005a; Looper et al., 2009). Our results suggest grazing E+ tall fescue prior to conception will decrease reproductive performance. Artificial insemination pregnancy rates (d 30) would suggest that grazing E+ fescue is more detrimental during or immediately prior to breeding. Natural service data supports this as TAI pregnancy rates were not affected by post-AI treatment, but cows grazing E+ post-AI had decreased pregnancy rates. The cause of decreased natural
service in addition to AI pregnancy rates (d 60 and 130) is uncertain due to natural
service insemination timing relative to treatment initiation; however, results would
suggest grazing E+ prior to conception is more detrimental to pregnancy establishment
and maintenance than grazing E+ post conception. Therefore, to prevent detrimental
reproductive effects of E+ grazing on cattle, open cows should be managed on nontoxic
forages. Once pregnancy is established, these data suggest no negative effects on
pregnancy maintenance in cows due to E+ grazing.

**Calf Performance.** There was no effect ($P > 0.05$) of E+ grazing pre-AI on
adjusted weaning weights or ADG compared with O pre-AI. Calves grazing E+ post-AI
had decreased ($P < 0.05$) adjusted weaning weights (Figure 3) and ADG (Figure 4).
Watson et al. (2004) reported ADG of 0.97 and 1.15 kg/d for E+ and novel endophyte,
respectively, for a difference of 15.6%. Similarly, Peters et al. (1992) reported 0.72 and
0.89 kg/d for E+ and endophyte-free fescue, respectively, for a difference of 11.2%. Our
data show an approximate 9.7% decrease in ADG of E+ calves compared with O post-AI.
For weaning weights adjusted to an average calf age of 205d, we found an approximate
7.5% difference between body weights of E+ and O calves post-AI. Similarly, Watson et
al. (2004) and Peters et al. (1992) reported a decrease of approximately 11% and 10%,
respectively, in E+ calves compared to calves on non-toxic forages.

In conclusion, we have verified our system of inducing fescue toxicosis in our
beef cattle herd as evidenced by depressed PRL and P4 concentrations. We also
confirmed our hypothesis that 2- and 3-yr old beef cattle grazing E+ prior to or switched
to E+ at insemination would exhibit lower pregnancy rates than cattle grazing other
grasses. However, we did not find an interaction of main effects E+ and O which would
have allowed us to more accurately determine if E+ was negatively affecting oocyte quality, fertilization processes, or early embryonic development. Grazing toxic tall fescue prior to and during the breeding season decreases pregnancy rates, but removing cattle from toxic tall fescue prior to or during the breeding season modulates these negative effects. Specific mechanisms by which E+ lowers pregnancy rates remain to be identified and additional research is needed.
Figure 1. Serum prolactin concentrations of cattle grazing endophyte-infected (E+) or nontoxic forages (O) pre-artificial insemination (AI) and post-AI (year 1, d 10; year 2, d 30) relative to timed AI (d 0). *Indicates significant difference ($P < 0.05$) in Prolactin concentration both pre and post AI treatment.
Figure 2. Cumulative pregnancy rates following timed AI (1\textsuperscript{st} pregnancy diagnosis on d 30) and natural service (2\textsuperscript{nd} and final pregnancy check d 60 and 130 respectively) for cows grazing toxic fescue (E+) pre- and post-AI (E+E+), E+ pre-AI and nontoxic forages (O) post-AI (E+O), O grasses pre-AI and E+ post AI (OE+), and O grasses (OO) pre- and post-AI.
Figure 3. Adjusted 205 d weaning weight for calves grazing toxic tall fescue (E+) or nontoxic (O) forages in the pre- and post-AI periods. *Within a grazing period (pre- and post-AI), calves grazing O differed ($P < 0.05$) from calves grazing E+. 
Figure 4. Average daily gain (ADG) for calves grazing toxic tall fescue (E+) or nontoxic (O) forages in the pre- or post AI periods. *Within a grazing period (pre- and post-AI), calves grazing O differed ($P < 0.05$) from calves grazing E+. 

Calf ADG (kg/d)

<table>
<thead>
<tr>
<th></th>
<th>Pre-AI</th>
<th>Post-AI</th>
</tr>
</thead>
<tbody>
<tr>
<td>E+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>O</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 1. Cumulative pregnancy rates for cows that grazed toxic tall fescue (E+) or other non-toxic forages (O; bermudagrass and annual ryegrass) pre- and post-AI.

<table>
<thead>
<tr>
<th>Pregnancy Diagnosis</th>
<th>E+ Pre-AI</th>
<th>O Pre-AI</th>
<th>E+ Post-AI</th>
<th>O Post-AI</th>
<th>SEM</th>
<th>Pre-AI</th>
<th>Post-AI</th>
<th>Pre * Post AI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st d 30</td>
<td>41.9&lt;sup&gt;b&lt;/sup&gt; (25/58)</td>
<td>62.8&lt;sup&gt;a&lt;/sup&gt; (34/56)</td>
<td>50.7 (31/62)</td>
<td>54.1 (28/52)</td>
<td>6.5</td>
<td>0.033</td>
<td>0.720</td>
<td>0.781</td>
</tr>
<tr>
<td>2nd d 60</td>
<td>55.0&lt;sup&gt;b&lt;/sup&gt; (30/58)</td>
<td>77.0&lt;sup&gt;a&lt;/sup&gt; (42/56)</td>
<td>55.09&lt;sup&gt;b&lt;/sup&gt; (35/62)</td>
<td>76.9&lt;sup&gt;a&lt;/sup&gt; (43/52)</td>
<td>6.4</td>
<td>0.016</td>
<td>0.013</td>
<td>0.966</td>
</tr>
<tr>
<td>Final d 130</td>
<td>69.1&lt;sup&gt;b&lt;/sup&gt; (38/58)</td>
<td>87.4&lt;sup&gt;a&lt;/sup&gt; (48/56)</td>
<td>66.8&lt;sup&gt;b&lt;/sup&gt; (44/62)</td>
<td>89.8&lt;sup&gt;a&lt;/sup&gt; (46/52)</td>
<td>5.8</td>
<td>0.031</td>
<td>0.008</td>
<td>0.748</td>
</tr>
</tbody>
</table>

<sup>ab</sup> Rows within Pre- or Post-AI, lacking a common superscript differ (*P < 0.05*)
Table 2. Independent pregnancy rates for cows that grazed toxic tall fescue (E+) or other non-toxic forages (O; bermudagrass and annual ryegrass) pre- and post-AI.

<table>
<thead>
<tr>
<th>Pregnancy Diagnosis</th>
<th>Pre-AI</th>
<th>Post-AI</th>
<th>SEM</th>
<th>Pre-AI P-value</th>
<th>Post-AI P-value</th>
<th>Pre * Post AI P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E+</td>
<td>O</td>
<td>E+</td>
<td>O</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1&lt;sup&gt;st&lt;/sup&gt;, d 0</td>
<td>41.9&lt;sup&gt;b&lt;/sup&gt; (25/58)</td>
<td>62.9&lt;sup&gt;a&lt;/sup&gt; (34/56)</td>
<td>50.7 (31/62)</td>
<td>54.1 (28/52)</td>
<td>7.0</td>
<td>0.033</td>
</tr>
<tr>
<td>2&lt;sup&gt;nd&lt;/sup&gt;, d 1 - 30</td>
<td>31.1 (9/37)</td>
<td>40.9 (10/24)</td>
<td>15.8&lt;sup&gt;b&lt;/sup&gt; (4/33)</td>
<td>56.1&lt;sup&gt;a&lt;/sup&gt; (15/28)</td>
<td>8.5</td>
<td>0.335</td>
</tr>
<tr>
<td>Final, d 30 - 60</td>
<td>32.5 (9/28)</td>
<td>58.2 (7/14)</td>
<td>31.5 (9/29)</td>
<td>59.3 (7/13)</td>
<td>13.00</td>
<td>0.189</td>
</tr>
</tbody>
</table>

<sup>a-b</sup> Rows within Pre- or Post-AI, lacking a common superscript differ (P < 0.05)
LITERATURE CITED


Caldwell, J., K. Coffey, C. West, J. Philipp, J. Jennings, D. Hubbell et al. 2010. Performance by spring and fall-calving cows grazing with full access, limited access, or no access to wild-type endophyte-infected fescue. Arkansas Animal Science Department Report. : 10.


Hoveland, C. S. 1993. Economic importance of *Acremonium* endophytes. Agriculture Ecosystems and Environment. 44(3)


CHAPTER 4: INVESTIGATING MECHANISM OF FESCUE TOXICOSIS IN BEEF CATTLE

PROJECT SUMMARY

Toxic tall fescue (E+) is the predominant cool-season perennial forage in the Southeastern U.S. and causes detrimental effects on reproductive and growth performance in beef cattle. Decreased pregnancy rates have been documented with E+ exposure, but the underlying mechanisms of how or at what stage of the reproductive cycle E+ negatively impacts male and female reproductive performance is unknown. In addition to unknowns associated with E+ exposure on reproduction, assessment of oocyte and embryo quality is currently based on subjective, visual appraisal. The underlying gene expression and metabolism associated with competent oocytes and embryos is not known. Specific aims set forth within this proposal are designed to establish an objective quality assessment matrix for oocyte and embryo evaluation, determine changes in gene expression and quality of oocytes, and evaluate embryo quality and pregnancy establishment potential of embryos due to E+ exposure in beef cattle. My hypothesis is that gene expression will differ in oocytes and embryos collected from cattle grazing E+ compared with those from cattle grazing non-toxic forages. Differences identified in gene expression will be associated with decreased embryo quality and subsequent implantation rates. Since E+ fescue is such a large part of most cow-calf grazing systems in the Southeastern U. S., further investigation to determine the timeframe in which E+
impacts pregnancy rates can lead to improved grazing management strategies to minimize decreased animal reproductive performance.
PROJECT NARRATIVE

Introduction

My long-term goal is to identify and understand mechanisms that result in reproductive failure. Short-term goals include the points of impact of toxic tall fescue on decreased reproductive performance in beef cattle. A thorough understanding of decreased reproductive success in cattle is key to increasing production efficiency of beef cattle in the United States. Reproduction is the limiting factor of profitability and production efficiency of a beef cattle operation (Dickerson, 1970; Dziuk and Bellows, 1983) and tall fescue \textit{[Schedonorus phoenix (Scop.) Holub]} is the predominant cool-season perennial forage in much of the Southeast and Mid-Atlantic regions of the U.S., with 8.5 million cattle grazing wild-type endophyte infected (E+) fescue. The estimated economic impact of E+ in beef cattle exceeds $600 million annually (Hoveland, 1993). Based on inflation from 1993 to 2012, E+ negatively impacts the beef industry at close to $1 billion annually. Decreased reproductive performance in cattle grazing E+ fescue has been documented (Schmidt et al., 1986; Gay et al., 1988; Clay and Scharld, 2002); however, specific mechanisms of how or at what stage of the reproductive cycle toxic fescue negatively impacts male and/or female reproductive performance is unknown. Toxic tall fescue is a vital tool for beef production in the U.S. and renovation costs from E+ pastures to novel endophyte free cultivars will most likely remain cost prohibitive. Therefore, research focusing on the management strategies to alleviate deleterious effects on reproduction and performance is greatly needed to assist producers with management decisions.
The literature is unclear as to whether E+ negatively impacts gamete development (male and/or female), fertilization, or early embryonic development. My previous results show that E+ exposure prior to insemination decreases pregnancy rates in 2 and 3 yr old beef cattle; however, E+ exposure immediately following insemination does not affect pregnancy rates. This suggests that oocytes exposed to E+ may be developmentally compromised. I hypothesize that gene expression will differ in oocytes collected from cattle grazing E+ compared with oocytes from cattle grazing non-toxic forages and can potentially be used as a quality assessment tool. Differences identified in gene expression may be associated with key reproductive events and also decreased embryo quality and subsequent implantation rates. With this knowledge, we will be able to identify stages of reproduction that are affected by fescue toxicosis.

Specific Aims

1) **Establish a quality assessment matrix based on gene expression for oocytes and embryos collected from beef cattle.** Embryo quality is historically assessed by visual appraisal; however, a quantitative quality matrix will be developed in this study. We will correlate visual appraisal with gene expression and fertility as a measure of quality.

2) **Determine changes in gene expression and quality of oocytes due to toxic tall fescue (E+) exposure in beef cows.** Cows will graze E+ and non-toxic forages. Gene expression from cumulus cells of oocytes collected from cows will be compared between the 2 grazing treatments and evaluated for quality.
3) Evaluate embryo quality and pregnancy establishment potential of embryos collected from cattle grazing toxic tall fescue (E+). Cows will be exposed to E+ and non-toxic forages and inseminated with semen collected from bulls fed E+ or non-toxic fescue (E-) seed. Collected embryos will be evaluated for quality and subsequent pregnancy rates to identify the predominant source of decreased reproductive performance in E+ cattle.
**Preliminary Results**

Our lab has shown successful induction of fescue toxicosis through grazing E+ pastures in heifers, 2- and 3-yr old cows, and feeding E+ seed to yearling beef bulls. Figure 1 represents serum PRL concentrations of cattle grazing E+ and other forages. Decreased prolactin levels are consistent with signs of fescue toxicosis (Elsasser and Bolt, 1987; Schillo et al., 1988). Grazing E+ prior to timed artificial insemination (TAI) decreased pregnancy rates in beef cows (Burns et al., 2012). In addition, grazing E+ post-TAI decreased adjusted weaning weights of calves. Due to the prevalence of E+ and cost associated with replacing E+ with alternate non-toxic forages, management strategies need to be developed in order to utilize current E+ pastures while relying on non-toxic novel endophyte-infected fescue (NE+) for grazing during critical times of the year. These strategies need to be based on the biology of when and how E+ negatively impacts reproduction.

Currently, we do not understand when the critical time or mechanism by which E+ exerts its deleterious effects on reproduction. Figure 2 shows pregnancy rates of cattle exposed to E+ both pre- and post-TAI (E+E+), E+ pre- and other forages (O) post-TAI (E+O), O pre- and E+ post-TAI (OE+), and O pre- and post-TAI (OO). Natural service pregnancy rates, indicated by 2nd, and final pregnancy checks suggest E+ impacts reproduction prior to insemination. It becomes very clear that E+ fescue negatively impacts reproductive performance in beef cattle. The current specific aims will address...
the timing of these negative impacts in order to increase animal reproduction efficiency
while grazing E+ tall fescue.
Figure 1. Serum prolactin concentrations of cattle grazing endophyte-infected (E+) or nontoxic forages (O) pre-artificial insemination (AI) and post-AI (year 1, d 10; year 2, d 30) relative to timed AI (d 0). *Indicates significant difference ($P < 0.05$) in Prolactin concentration both pre- and post-AI treatment
Figure 2. Cumulative pregnancy rates following timed AI (1<sup>st</sup> pregnancy diagnosis on d 30) and natural service (2<sup>nd</sup> and final pregnancy check d 60 and 130 respectively) for cows grazing toxic fescue (E+) pre- and post-AI (E+E+), E+ pre-AI and nontoxic forages (O) post-AI (E+O), O grasses pre-AI and E+ post-AI (OE+), and O grasses (OO) pre- and post-AI.
Approach

**Specific aim 1:** Establish a quality assessment matrix based on gene expression for oocytes and embryos collected from beef cattle.

*Rationale:* Historically, embryo and oocyte quality assessment is based on a visual observation of cell and embryo structure and competence (Robertson and Nelson, 1998). Visual appraisal has been fairly successful when comparing embryo grade with pregnancy rates; however, an ability to objectively evaluate oocytes and embryos may lead to higher *in vitro* fertilization (IVF) rates and increased *in vivo* pregnancy rates. Current research suggests that amino acid turnover may in fact be a good indicator of oocyte competence (Hemmings et al., 2012). Research has also investigated expression of genes in cumulus cells that may act as a marker of competence in oocytes (Caixeta et al., 2009). Ghanem et al. (2011) has shown that certain genes are up- or down-regulated in embryos that will not produce a viable pregnancy in a recipient cow regardless of culture environment. Expression of reference genes changes over time in maturing oocytes and developing embryos; therefore, it is essential that RNA is isolated and compared from cells at the same stage of development (Bettegowda et al., 2006). Visual observation, amino acid profile, and gene expression will be correlated in one study to develop a quantitative oocyte and embryo quality matrix.

*Experimental Design:* Cumulus-oocyte complexes (COC) will be collected from ovaries obtained from an abattoir and matured *in vitro*. Cumulus-oocyte complexes will be cultured in *in vitro* maturation (IVM) media for 18 h (described below). At 18 h, IVM
media will be replaced with an amino acid profiling medium that contains reduced amino acids by substituting 85% of tissue culture Medium 199 with Earle’s Balanced Salt Solution. After 6 h, amino acid profiling medium will be collected and retained for amino acid analysis using high performance liquid chromatography (HPLC). Amino acid turnover in in vitro maturation (IVM) media will be used as a predictor of competent oocytes. The COC will be subjected to IVF and cumulus cells will be isolated 18 to 22 h later. Cumulus cell isolation and RT-PCR will be used for gene expression analysis of oocyte competence. Cleavage rates and blastocyst formation will be assessed as a measure of competence. A subset of embryos will be randomly selected for gene expression analysis and remaining embryos will be frozen and stored in liquid N₂. Visual grade and amino acid turnover will be compared to gene expression data to develop an objective grading matrix for competent oocytes and embryos. Data will be analyzed and statistical contrast developed in an effort to systematically evaluate oocyte and embryo competence. Based on parameters in the model, evaluation indexes will be correlated back to oocyte and embryo quality.

Expected results and concerns: Due to a very small amount of starting material and elusiveness of a stable reference gene, gene expression analysis of oocytes and developing embryos is challenging. However, differences in gene expression from oocytes and embryos exposed to different environmental conditions have been observed (Khosla et al., 2001). Using visual appraisal, amino acid profile, and gene expression analysis, I am confident we will develop an objective method for assessing oocyte and embryo integrity. The number of oocytes and embryos needed to perform this study will
be a challenge also. Therefore, we expect to compensate low IVF efficiency and large number of oocytes and embryos required with our initial animal number.

**Specific aim 2:** Determine changes in gene expression and quality of oocytes due to toxic tall fescue (E+) exposure in beef cows.

**Rationale:** Fescue effects can be alleviated using novel endophyte (NE+) varieties, however, currently replacing existing E+ stands with NE+ is not cost effective for a large portion of producers. Current research from our lab would indicate a 35% decrease in pregnancy rate when cattle are grazing E+ tall fescue during the breeding season (Burns et al., 2012a). However, it is currently unknown if the decreased pregnancy rates are due to oocyte quality, embryo quality, or uterine competence. Evaluation of oocyte gene expression as a measure of oocyte competence will lead to a better understanding of the basic mechanisms causing decreased fertility in cattle grazing E+ tall fescue. Understanding the timing of these negative impacts is crucial to developing and implementing management strategies to improve the efficiency of southeastern beef production in the U.S. Unfortunately, it is difficult to detect early pregnancy and monitor fertilization and embryo development in vivo. Therefore, *in vitro* fertilization has been used successfully to monitor processes involving oocyte maturation, fertilization, and embryo development.

**Experimental Design:** Twenty Angus and Angus-crossbred cows will be grazed on E+ (>75% infected, n = 10) tall fescue or other forage (O, n = 10) for at least 60 d prior to being used for oocyte collection. Oocytes will be collected twice weekly, using
ultrasound-guided ovum pick up (OPU). Cumulus-oocyte complexes will be matured in vitro and subjected to IVF. Maturation of the oocyte will take place over an 18 h period using IVM media (see Experimental Methodologies). Oocytes will be incubated for 6 h in amino acid profiling media to profile amino acid turnover using HPLC (Hemmings et al., 2012). Following IVF, presumptive zygotes will be denuded of cumulus cells and evaluated for cleavage and blastocyst formation rates. Blastocysts will biopsied, re-expanded, and cryopreserved for future implantation in recipients or directly implanted in recipients. Gene expression of cumulus cells and biopsies will be analyzed using RT-qPCR for histone H2A (H2A), follicle-stimulating hormone receptor (FSHR), Epidermal growth factor receptor (EGFR), and growth hormone receptor (GHR).

*Expected results and concerns:* Reproductive performance is decreased when cattle graze E+ tall fescue. Oocyte competence may be the cause of this decreased performance. I expect to find differential gene expression between cumulus cells of oocytes collected from cattle grazing E+ and O. Identification of marker genes associated with fescue toxicosis may correlate with the objective quality assessment matrix developed in Specific Aim 1. In addition, differential expression of histone H2A (H2A) has been identified in the oocyte and correlated with genes in cumulus cells (FSHR, EGFR, and GHR) as an indicator of oocyte competence (Caixeta et al., 2009), and may be affected with E+ grazing treatment.
Specific aim 3: Evaluate embryo quality and pregnancy establishment potential of embryos collected from cattle grazing toxic tall fescue (E+).

Rationale: Decreased female reproductive performance in beef cattle is well-documented due to E+ exposure (Schmidt et al., 1986; Gay et al., 1988; Clay and Schardl, 2002; Burns et al., 2012a); however, male reproductive performance and additive effects of both male and female exposure to E+ is less known. Embryo quality has a significant impact on pregnancy rate when referring to assisted reproductive techniques involving embryo transfer (Hasler, 2001). Determination of embryo quality through gene expression is an effort to elucidate causes of decreased pregnancy rates in cattle gazing E+ tall fescue. The ultimate successful outcome for an embryo would be establishment of pregnancy in a recipient cow. Therefore, embryos collected from donors grazing E+ tall fescue and non-toxic forages will be evaluated for their capacity to establish pregnancy.

Experimental Design: Twenty Angus and Angus-crossbred cows will be grazed on E+ (>75% infected, n = 10) tall fescue or other forage (O, n = 10) for at least 60 d prior to being used as donors for embryo transfer. Cows will be superstimulated to produce multiple ovulations within a single estrous cycle. Half of the donors in each grazing treatment will be inseminated with frozen-thawed semen collected from bulls fed a control, endophyte-free (E-) seed. The remaining donors will be inseminated with semen collected and frozen from E+-fed bulls. Bull diets with E+ seed were formulated to contain 0.8 ppm ergovaline and ergovalanine on a dry matter basis. Embryos collected
from donor cattle will represent a 2x2 factorial design consisting of E+ donors bred to E+ bulls (E+E+) and E- bulls (E+E-), and O donors bred to E+ bulls (OE+) and E- bulls (OE-). Embryos will be collected using the transcervical flush method. Donors will be superstimulated and inseminated repeatedly with semen of alternating treatment groups. Upon collection embryos will be visual assigned a grade, and then a subset of embryos will be selected to be biopsied for gene expression. Embryos will be re-expanded after the biopsy process and implanted with the remaining embryos. Embryos will be transferred into valid recipients, which have not been exposed to E+ and pregnancy will be assessed on d 23 after transfer. Pregnancy will be confirmed on d 53 after transfer.

*Expected results and concerns:* Embryo competence and ability to establish and maintain pregnancy is negatively affected by grazing E+ tall fescue. Superstimulation and successful embryo recovery can be challenging due to animal-to-animal variation. Protocols will be optimized for each donor in order to maximize donor ovulations and embryo retrieval. I expect to identify specific genes that correlate to general embryo quality. By completing specific aim 1 and 2 we will have a more thorough understanding of the negative effects and whether those effects are pre-fertilization, post-fertilization or a combination of the two.
**Experimental Methodologies**

*Oocyte maturation/IVF:* Cumulus oocyte complexes (COC) will be collected from an abattoir or cow donors twice-weekly using ultrasound guided transrectal ovum pick up (Merton et al., 2003). Oocytes will be matured and IVF procedures performed according to Lawrence et al., (2004). Briefly, oocytes will be cultured in tissue culture Medium 199 supplemented with 10% fetal bovine serum (FBS), 0.03 µg/mL LH and 5.0 µg/mL FSH over a 24 h period and incubated in a humidified environment at 38.5°C with air containing 5.5% CO₂ (Lawrence et al., 2004). Oocytes will be washed 2x using synthetic oviduct fluid (SOF) supplemented with HEPES medium. Initial classification of oocytes will be based on amino acid profiles of maturation fluid (Hemmings et al., 2012). Motile sperm will be obtained using the Percoll method (Parrish et al., 1995) and IVF will be performed using frozen-thawed semen. Cumulus-oocyte complexes will be incubated for 18 to 22 h with frozen-thawed semen. Cumulus cells will be isolated post-fertilization from individual oocytes, frozen, and stored at -80°C until RNA isolation. Cleavage rates of presumptive zygotes will be evaluated 2 d post-IVF (d 0). Embryos will continue to be cultured in SOF media containing FBS and amino acids and evaluated on d 6, 7, and 8 for blastocyst formation. Blastocysts will be harvested in-entirety or biopsied using micromanipulation for gene expression analysis. Briefly, one manipulator will hold the embryo in place while the other is equipped with a small glass needle to penetrate the zona and remove 10 to 20% of the embryonic mass. Biopsies will be handled using a pipet with aerosol barrier tip. Biopsied embryos will be cultured for 2 h to allow for re-expansion and transferred to recipients or frozen in liquid N₂ for future
implantation studies or analysis. Embryos will be frozen using the open pulled straw method previously defined in Vajta et al., (1998) Briefly, embryos will be incubated in medium containing 7.5% ethylene glycol and 7.5% dimethyl sulfoxide for 3 min. Secondly, embryos are moved to a medium containing 16.5% ethylene glycol and 16.5% dimethyl sulfoxide for 25 s. Following this step, the straws are placed directly in liquid nitrogen.

**Donor synchronization and flushing:** Donors will be synchronized and superstimulated using a combination of GnRH, progesterone releasing device (CIDR), and FSH. Cattle will be visually observed for estrus and inseminated once approximately 12 and 24 h after standing estrus. *In vivo*-produced embryos will be collected from donors on d 7 post-insemination by flushing the uterus transcervically. Embryos will be washed and placed in a holding medium until located using high power microscopy. *In vivo*-produced embryos will then be biopsied for gene expression analysis, re-expanded, and cryopreserved using the open pulled straw method described above or directly implanted in recipients. Cryopreserved embryos will remain frozen in liquid nitrogen until used for implantation in recipients or further gene expression analysis.

**Gene Expression using Real time RT-qPCR:** Total cellular RNA is isolated from cells using the mirVana microRNA Isolation kit (Ambion, Austin, TX) according to manufacturer’s instructions. Quality is assessed using Nanodrop 2000 Spectrophotometer (Thermo Fisher Scientific, Inc., Waltham, MA) and Agilent Bioanalyzer 2100. All tcRNA samples used in real time qPCR will have a 260:280
absorbance ratio $\geq 1.7$ on the Nanodrop and RNA integrity number $\geq 7.0$ (1.0 to 10.0 scale) using Agilent RNA 6000 Nano or Pico kit. Pooled samples will be sent off for commercial microarray analysis. Jones et al. (2004) had success in determining gene expression differences of luteal tissue in cattle exposed E+ and E- using a commercially available interspecies microarray. For validation of microarray results, Superscript III reverse transcriptase (Invitrogen Corp., Carlsbad, CA) is used to synthesize first strand cDNA. Real time qPCR is conducted using an Eppendorf MasterCycler ep realplex (Westbury, NY) with the QuantiTect SYBR Green RT-PCR Two Step Kit (Qiagen, Valencia, CA) according to the manufacturer’s directions. Previously, 2 genes, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and $\beta$-actin, were evaluated as housekeeping genes for data normalization in adipose tissue (Goldstein et al., 1979). For embryos and cumulus cells, an appropriate normalization gene will need to be determined using BESTKEEPER program (http://www.gene-quantification.info). The program determines the most stable housekeeping gene to be used for normalization by repeated pair-wise correlation and regression analysis (Pfaffl et al., 2002). Both GAPDH and $\beta$-actin exhibited a correlation coefficient of 0.99 ($P < 0.001$) in the analysis and were suitable for data normalization. Primers for bovine mRNA are designed using Primer 3 software (http://frodo.wi.mit.edu/primer3/). Primer sets for genes involved in fatty acid biosynthesis including acetyl-CoA carboxylase (ACC), fatty acid synthase (FASN), fatty acid elongase (ELOVL) -5 and -6, stearoyl-CoA desaturase (SCD1), sterol regulatory element binding protein-1c (SREBP), and carnitine palmitoyl-transferase 1A (CPT1A) were first evaluated according to Duckett et al. (2009) to verify identity. Genes of
interest for this work may include SCD1, as its expression was reduced in adipose tissue of bulls consuming E+ seed (Burns et al., 2012b). Also, embryo competency to produce a viable pregnancy is positively associated with placenta-specific 8 (PLAC8) and negatively associated with isolate 405 mitochondrion (FL405) and heat-shock 60 kDa protein 1 (HSPD1; Ghanem et al., 2011b). Other genes identified by microarray may also be targets for further gene expression analysis.

**HPLC amino acid profiling:** Aliquots of amino acid profiling medium will be thawed at room temperature, diluted 1:12.5 in HPLC-grade water, and analyzed using a Shimadzu HPLC system as described by Hemmings et al. (2012). In brief, samples will be reacted with O-phthaldialdehyde, standardized with D-α-aminobutyric acid, and run on an octadecyl silica column. Solvent A [80% (vol/vol) sodium acetate (pH 5.9), 20% (vol/vol) methanol and 5 ml/L tetrahydrofuran] and solvent B [80% (vol/vol) methanol, 20% (vol/vol) sodium acetate (pH 5.9), and 5 ml/L tetrahydrofuran] are used at a flow rate of 1.2 ml/min with an initial ratio of 100:0 (solvent A: solvent B) and final ratio of 0:100 (solvent A: solvent B). Differential elution times at an emission wavelength of 450 nm are compared with known standards to confirm identity. Net appearance and disappearance of amino acids will be calculated as pmol/embryo per h compared with blank media.


Hoveland, C. S. 1993. Economic importance of *Acremonium* endophytes. Agriculture Ecosystems and Environment. 44(3)


