Abstract

POOLE, REBECCA KYLE. Evaluation of Chronic Ergot Alkaloid Exposure and Various Mitigation Strategies on Performance, Reproduction, and Immunity in Beef Cattle. (Under the direction of Dr. Daniel H. Poole).

Fescue toxicosis is a multifaceted syndrome common in cattle consuming ergot alkaloids produced by endophyte (*Epichloë coenophiala*)-infected tall fescue [*Lolium arundinaceum* (Schreb.) Darbysh.]. Detrimental symptoms of this syndrome include reduced animal weight-gains, intolerance to high environmental temperature (heat stress), vasoconstriction, endocrine imbalance, and reduced conception rates. Together, these result in nonproductive animals and producers are burdened with reduced profitability. Strategies have been made to negate symptoms of fescue toxicosis, however these strategies are often not cost effective and only act as temporary solutions. Therefore, a better understanding of the physiological actions of ergot alkaloids is crucial for the development of long-lasting solutions and improvements in cattle performance. Chapter 2 addresses the interaction of ergot alkaloids and heat stress on reproductive performance. Angus and Senepol crossbred heifers were genotyped either wildtype (W) or slick mutation (S; heat tolerant) and assigned to a diet with or without ergot alkaloids (EI vs. EF, respectively). Only EI-W heifers had a reduced number of preovulatory follicles (≥9 mm) following estrous synchronization and all were confirmed open 30 d post-insemination ($P<0.05$). These results demonstrate that the slick mutation appears to aid in offsetting the heat stress related symptoms associated with fescue toxicosis and improves reproductive performance. Chapters 3, 4, and 5 address the interaction of ergot alkaloids and protein supplementation on hemodynamics, immunity, and subsequent feedlot performance of stocker steers. In Chapter 3, Angus steers were weaned and assigned to either EF or EI diets with either 14% or 18% crude protein. The EI-14 steers had elevated skin surface temperatures and reduced respiration rates ($P<0.05$), indicating

the ability to properly dissipate excess body heat. Whereas the EI-18 steers exhibited reduced hematocrit, slower heart rates, and vasoconstriction of the caudal blood vessels ($P<0.05$), all common symptoms of fescue toxicosis. Together, demonstrating exposure to ergot alkaloids negatively impacts hemodynamic responses and supplemental protein provided minimal benefit. In Chapter 4, regardless of protein supplementation all EI steers had elevated concentrations of pro-inflammatory cytokines (TNF-α, IFN-α, IFN-γ, IL-1α), anti-inflammatory cytokines (IL-2, -13, -15, -21, -36), and chemokines (CCL2, CCL4, MIG) when compared to EF steers ($P<0.05$). Additionally, EI-14 steers had greater concentrations of VEGF-A and IGF-1 ($P<0.05$). These are growth factors involved with angiogenesis and would indicate peripheral vasodilation, which would be in agreement with the skin surface temperature results in Chapter 3. Based on these results, stocker steers chronically exposed to ergot alkaloids have a hyperactive innate immune response and protein supplementation had minimal impact to mitigate this response. Finally in Chapter 5, steers were followed through the feedlot phase where growth performance and carcass traits were evaluated. The EI-14 steers had a greater ADG and feed efficiency during the growing phase (first 52 d in feedlot; $P<0.05$). This indicates compensatory gain which is common in feedlot cattle that previously grazed endophyte-infected tall fescue. Ultimately, previous exposure to ergot alkaloids did not affect post-harvest carcass characteristics ($P>0.05$). Collectively, the results presented in this dissertation indicate that chronic exposure to ergot alkaloids negatively impact performance, reproduction, and immunity; and there are some mitigation strategies that can provide beneficial outcomes.

Evaluation of Chronic Ergot Alkaloid Exposure and Various Mitigation Strategies on Performance, Reproduction, and Immunity in Beef Cattle

by
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Dedication

To my great-grandmother, grandmother, and mother. Strong women who showed me the value in education.

Biography

Rebecca "Becky" Kyle Poole was born on July 23, 1992, to Bill and Donna Poole. She grew up in the small, rural town of Chocowinity, North Carolina with a younger sister, Sarah, and two older half-brothers, Jeff and David. Despite having a non-agricultural childhood, she always wanted to go to NC State and pursue a career in veterinary science. She graduated from Southside High School in May of 2010 and started her undergraduate program at North Carolina State University in August of 2010, majoring in Animal Science.

After her sophomore year at NC State, she had taken Reproductive Physiology with Dr. Daniel Poole and decided to alter career paths. In the summer of 2013, she took Dr. Charlotte Farin's Goat Artificial Insemination Short Course and after this experience, she assisted with various research projects in Dr. Farin's lab. These projects in conjunction with her taking every repro-focused course offered by the department, she developed a strong desire to continue to learn about reproduction in livestock species. In talking with Dr. Farin about career options and the possibility of graduate school, she was put in contact with Dr. Alan Ealy at Virginia Tech.

Becky graduated from North Carolina State University in May of 2014 with a Bachelor of Science in Animal Science. Following graduation from NC State, she went to Virginia Tech to obtain a Masters in Animal and Poultry Sciences under the direction of Drs. Michelle Rhoads and Kiho Lee. While at Virginia Tech, her thesis project focused on mechanisms associated with lower fertility as a result of maternal obesity. Utilizing pigs as a model, she explored how high saturated fat and fructose consumption facilitated obesity-induced infertility, specifically using an *in vitro* culture system to observe a disruption in early embryonic development. In addition to her work, she assisted a fellow graduate student in Dr. Alan Ealy's lab which used a sheep model to investigate the impact of maternal obesity on epigenetic regulation of reproduction. In June of

2016, she obtained her Master of Science in Animal and Poultry Sciences with a concentration in reproduction. Following graduation, Becky returned to North Carolina State University to begin her doctoral education in Animal Physiology under the direction of Dr. Daniel Poole (no relation). While at NC State, she worked on many projects related to fescue toxicosis and its detrimental effects on beef cattle reproduction and immunity. Additionally, she has been greatly involved in the annual breeding projects overseen at NCSU's Butner Beef Cattle Field Laboratory and NCDA's Upper Piedmont Research Station. The premise of these projects is to conduct research relevant to producers and help incorporate these strategies in their herds, in the hope that they will become more efficient and profitable. After graduation, she plans to continue her multidisciplinary approach to understanding the regulation of mammalian fertility in a post-doctoral position.

Acknowledgments

The successful completion of this degree would not have been possible without the encouragement and tireless efforts of many individuals. First and foremost, I would like to express my sincere gratitude to Dr. Daniel Poole. I feel as if no amount of words can truly sum up my full appreciation, but I will give it a try. Thank you for providing me with the foundation to what I trust will be a successful and rewarding career in science. Through your endless support, guidance, and I am sure sometimes patience, you have substantially improved both my research and teaching abilities. Going above and beyond as a mentor, both professionally and personally, the time spent working together has become a relationship that will I cherish and will certainly last a lifetime. Additionally, I would like to thank my committee members: Drs. Charlotte Farin, Matt Poore, John Meitzen, and John Gadsby for providing their time, expertise, constructive criticism, and encouragement towards the progression and enhancement of my education.

It is probably now known that I have a slight obsession with the Harry Potter series. Having read this series many times, it is not just a story about good vs. evil, it also teaches a great deal about the value of friendship. Probably one of my favorite quotes from the series perfectly illustrates the bond shared between graduate students, "*We are only as strong as we are united, as weak as we are divided.*" Without a doubt, you all have made this process better and I am eternally grateful for all the assistance and friendships made throughout the years. I would like extend a special thank you to a few graduate students. To Kyle Mayberry and McKayla Newsome, you both were incredible lab mates and I am exceedingly thankful for your boundless help on these projects. Most importantly though, I am so thankful for the countless laughs, memories, and our friendship. I would also like to thank Jeff Wiegert, you push me to not only be a better scientist, but to be a

better person. Additionally, I would be remiss without extending my appreciation to the incredible undergraduate students, past and present, for their assistance with various aspects of these projects.

I owe a tremendous amount of thanks to the Butner Beef Cattle Field Lab crew for their assistance with the day to day management of the cattle used in these projects and making my job that much easier. Thank you to the Department of Animal Science, the College of Agriculture and Life Sciences, as well as the North Carolina Cattlemen's Association for their financial support. Additionally, I would like to thank Missy Lloyd for all of her assistance while validating the prolactin assay.

Finally, I wish to express my deepest gratitude to my family who have given me unconditional love and support throughout this entire process. You provided me with the greatest gift of all, you believed in me. "*Working hard is important. But there is something that matters even more: Believing in yourself.*"

Table of Contents

List of Tables

List of Figures

Introduction

Kentucky-31 tall fescue [*Lolium arundinaceum* (Schreb.) Darbysh.] is the most common cool-season perennial forage utilized by beef cattle producers due to its dependability and persistence (Burns and Chamblee, 1979; Ball et al., 2007). The majority of tall fescue has a symbiotic relationship with an endophyte (*Epichloë coenophiala*) that provide numerous agronomic benefits, however also produce ergot alkaloid compounds that are toxic to grazing animals and result in a condition known as fescue toxicosis (Lyon et al., 1986). This syndrome contributes to an estimated $1 to 2 billion annually in economic loss predominantly due to a reduction in conception rates and poor growth performance and health in calves, all which are vital to the productivity and profitability of the beef industry (Strickland et al., 2011; Kallenbach, 2015).

While tall fescue is economically important to the sustainability and prosperity of agriculture in the United States, the negative impact this forage has on beef cattle performance is an issue that requires a solution. Therefore, the goal of this research is to gain a better understanding of the physiologic and/or cellular actions of ergot alkaloids on 1) follicular and luteal development in heifers, 2) performance parameters in stocker steers, 3) immune responsiveness in steers post-vaccination, and 4) subsequent finishing growth and carcass traits of stocker steers; and additionally propose possible solutions to subdue the symptoms of fescue toxicosis and improve overall performance.

Chapter 1: Literature Review

1.1 Beef Cattle Production Systems

A variety of beef breeds are common in the United States including Angus, Hereford, Charolais, Simmental, and Brahman; with each providing several beneficial characteristics to the beef industry. British breeds, Angus and Hereford, originated in either England or Scotland respectively, were imported to North America in the 1800s, and known for retention of body fat reserves, early age of puberty, and longevity (Herring, 2014). Whereas continental European breeds, Charolais and Simmental, originated in either France or Switzerland respectively, were imported to North America in the early 1900s, and considered dual-purpose due to lean body composition and greater levels of milk production (Herring, 2014). Both British and continental European breeds are *Bos taurus*, however Brahman are imported Zebu (*Bos indicus*) cattle and popular in Southern United States because of their heat and parasite tolerance (Herring, 2014). While there is a diversity of cattle breeds in the United States, most cattle raised for meat production are actually crossbreds, with approximately 60% having some Angus influence (Drouillard, 2018).

Beef cattle production systems in the United States are categorized into three primary sectors: cow-calf, backgrounding-stocker, and feedlot operations. Similar to beef cattle breed distribution, geographical distribution of each sector is heavily influenced by many factors including environmental conditions, forage availability, cereal grain production, human population density, and available agricultural land area (Herring, 2014; Drouillard, 2018).

1.1.1 Cow-calf

The cow-calf sector can simply be defined as an operation that maintains a breeding herd of cows that each produce a calf annually, then typically sell the calves at or shortly after weaning

(Herring, 2014). While cow-calf operations are located across the United States, most are in the Southeast region because of the mild climate and abundant forage availability (McBride and Mathews, 2011). However, a limitation of this region is that there is a finite acreage available for grazing, thus most are smaller operations with an average herd size of 41 cows (McBride and Mathews, 2011; Herring, 2014). As a result, most cow-calf producers rely on outside sources of income in order to sustain the operation (USDA NASS, 2012).

The cow-calf sector can be further divided into seedstock or commercial segments. Seedstock operations are the purebred sector and the primary source of new genetics for the entire beef industry (McKinnon and Snodgrass, 2009). For most seedstock operations, the majority of income is acquired by selling genetically superior yearling bulls and producers will commonly register animals with a breed association (McKinnon and Snodgrass, 2009; Herring, 2014). Commercial cow-calf operations produce non-registered animals and constitute as the largest sector of the beef industry (McKinnon and Snodgrass, 2009; Herring, 2014). Typically, commercial operations will purchase a bull from a seedstock operation and maintain breeding females to produce an annual calf crop (Herring, 2014). Calves will be weaned at approximately 6 to 9 months of age and the majority will be sold to backgrounders (McBride and Mathews, 2011).

1.1.2 Backgrounding-stocker

Backgrounding refers to a brief transitional phase (typically 3 to 6 months) for substantial weight gain in calves between weaning and feedlot entry (Herring, 2014). Specifically, stocker operations are a type of backgrounding system that relies on an abundant source of high quality forage to grow calves (McKinnon and Snodgrass, 2009; Herring, 2014). Because both cow-calf and stocker operations predominantly utilize forage, some cow-calf operations will retain calves after weaning and continue grazing for 30 to 90 days before selling to a feedlot. However, forage

availability will determine the stocking capacity of pasture land and ultimately determine whether calves will be retained or sold at weaning (McBride and Mathews, 2011). Typically, due to limited pasture land available in the Southeast, most operations that retain calves are much larger and located in the Great Plains (McBride and Mathews, 2011; Herring, 2014).

One of the most important functions of a backgrounding system is proper management of calves through the stressful adjustment period of weaning, abrupt changes in diet, and transportation. In fact, during this transition period calves will be most susceptible to bovine respiratory disease (BRD) complex, and this is commonly referred to as "shipping fever" (McKinnon and Snodgrass, 2009; Herring, 2014). This disease affects the lower respiratory tract and is caused by four viral BRD pathogens including infectious bovine rhiotreachitis (IBR), bovine respiratory syncytial virus (BRSV), bovine viral diarrhea (BVD), and parainfluenza-3 (PI3; Herring, 2014). Additionally, a viral respiratory infection by one or combination of pathogens will result in animals being susceptible to secondary bacterial infections that typically result in bronchopneumonia (Cusack et al., 2003; Herring, 2014).

1.1.3 Feedlot

Cattle entering feedlot operations range from newly weaned calves to yearlings and this final stage of cattle production is referred to as either cattle feeding or finishing (McKinnon and Snodgrass, 2009). These operations are far more concentrated geographically when compared to cow-calf or stocker operations, with the overwhelmingly majority of feedlots being located in Nebraska, Texas, Kansas, Iowa, and Colorado, otherwise known as the "Corn Belt" (Drouillard, 2018). This region is desirable because the feeding industry has quick access to cheap grains, primarily corn, wheat, sorghum, and grain byproducts which are extensively used in finishing diets (Herring, 2014; Drouillard, 2018).

The goal of a feedlot operation is to feed at low-costs for a minimum of 90 days until it is believed that they will produce a satisfactory carcass quality and yield grade (McKinnon and Snodgrass, 2009). Quality and yield grades reflect very different carcass traits. Quality grades are determined based on the maturity of the animal at harvest and amount of intramuscular fat (i.e. marbling) present with Prime, Choice, and Select grades typically being used for retail and food service (Holland and Loveday, 2013). Yield grades are rated numerically from 1 to 5 and are an estimate of the relative amount of lean, edible meat from a carcass with these variables taken into account: amount of external fat, hot carcass weight (HCW), amount of kidney, pelvic, and heart (KPH) fat, and the area of the ribeye muscle (Holland and Loveday, 2013). Feedlots are rewarded for producing highly marbled beef, yet are discouraged from over-fattening and producing a carcass with a yield grade of 4 or 5 (Drouillard, 2018).

Many feedlot operations will typically purchase heavier cattle (from a backgrounding system) which will require less feeding time, however some have transitioned to receiving weaned calves from a wide range of geographic locations with unknown immune status (Herring, 2014). Consequently, cattle arriving at the feedlot have the added risk of contracting BRD due to pathogen exposure and disease susceptibility. During the feedlot phase, it is estimated that BRD accounts for 70-80% of morbidity and 40-50% of mortality, thus being the most significant health problem facing the U.S. beef cattle industry (Duff and Galyean, 2007; Hilton, 2014). A lack of immunity via failed vaccination and/or lack of natural exposure to these pathogens in the cow-calf phase increases the likelihood that cattle will develop BRD during the stocker or feedlot phase, thus determine the profit for stocker and feedlot operations (Duff and Galyean, 2007; Herring, 2014). Therefore, strides must be taken to ensure that calves develop a strong immune system during cow-calf production and carried through each sector of the beef production.

1.2 Kentucky-31 Tall Fescue

Tall fescue [*Lolium arundinaceum* (Schreb.) Darbysh.] is a cool-season perennial forage and perhaps the most important cultivated grass in the United States. Native to Europe, tall fescue was introduced to the United States in the 1800s, however it was not widely established until the 1940s. Kentucky-31 (KY-31) is variety of tall fescue that was observed by a University of Kentucky professor, Dr. E.N. Fergus, in 1931 (Hoveland, 2009). Dr. Fergus noted that this grass was able to thrive and withstand the harsh winter; and after rigorous testing in several locations, KY-31 was released as a cultivar of tall fescue in 1943 (Fergus, 1952). Over the years, cattle producers have shown preference towards KY-31 due to its dependability, adaptability to various soil types, ability to withstand drought conditions, and stockpiling capability, thus providing a viable grazing option for much of the year. Currently, KY-31 is estimated to be grown on 35 million acres of land, specifically throughout the lower Midwest and Southern regions of the United States (Burns and Chamblee, 1979; Strickland et al., 2009).

Cool-season forages, such as tall fescue, maximally grow during the spring and autumn months and growth ceases during the summer months, often referred to as the "summer slump" (Sleper and West, 1996). Typically, cool-season forages have reduced growth when temperatures rise above 25°C (Cooper and Tainton, 1968), however tall fescue is able to persist even when temperatures reach 35°C (Hannaway et al., 2009). This persistence is mainly associated with the amount of soil moisture. Tall fescue requires moist, well-drained soil for optimal growth, however it can survive in various type of soil drainage levels from poorly-drained to excessively-drained (Burns and Chamblee, 1979; Hannaway et al., 2009). Even during the hot summer months when there is high evaporative demand (relationship between soil water evaporation and amount of precipitation), tall fescue is able to survive and this is most likely due to its extensive root system

(Sleper and West, 1996). For these reasons, tall fescue is able to adapt and persist in the temperate, humid climate of the lower Midwest and Southern regions of the United States.

1.2.1 Endophyte and Symbiosis

The word "endophyte" is a generic term describing an organism (i.e. fungi) that lives within a plant or host. Clavicipitaceae are a family of fungi which include the species of *Epichloë* and its related *Neotyphodium* species; these species form an endophytic symbiosis with numerous grasses (Leuchtmann et al., 2014). In tall fescue, the endophyte was initially identified as *Epichloë typhina* (Bacon et al., 1977), however it was later renamed *Acremonium coenophialum* (Morgan-Jones and Gams, 1982). About a decade later, *Acremonium* fungi were reclassified using phylogenetics and ultimately renamed *Neotyphodium coenophialum* (Glenn et al., 1996). Finally, Leuchtmann et al. (2014) identified that *Neotyphodium* fungi must also include *Epichloë,* therefore the tall fescue endophyte is now recognized as *Epichloë coenophiala*.

The *Epichloë* species has produced a diversity of asexual forms, *Neotyphodium* species, which form a symbiotic relationship with cool-season grasses (Schardl, 1996). Within cool-season grasses, the fungal endophyte inhabits a nutrient-rich niche within the plant's interior known as the apoplasm. This area protects the endophyte and has relatively little competition from other microorganisms (Kuldau and Bacon, 2008). Furthermore, it has been suggested that the endophytic fungus develops a "defensive mutualism" with the host plant. Defensive mutualism typically involves the protection of the host plant by animals (i.e. ants), but this phenomenon is less common with fungi. However, the nature of the symbiotic relationship between *Epichloë coenophiala* and tall fescue can be classified as defensive mutualism (Clay, 1988).

Likewise, this symbiotic relationship benefits the host plant. For example, the endophytic fungus has induced adaptations within the host which allow for enhanced drought tolerance. When

compared to endophyte-free varieties of tall fescue, the endophyte infection allowed for improved water uptake from the soil due to an increase in root length and growth (Arraudeau et al., 1989; Bouton et al., 1993a; Malinowski et al., 1999). Endophyte-infected grasses are typically able to successfully outcompete non-infected grasses, and this is most likely due to morphological differences between the plants. These differences included greater number of tillers present (Hill et al., 1990) and as previously mentioned an extensive root system growth (Arraudeau et al., 1989; Bouton et al., 1993a; Malinowski et al., 1999). Additionally, because *Epichloë* fungi are seed-transmitted endophytes, they produce alkaloids that benefit the host plant against many pests. Loline (pyrrolizidine) alkaloids, such as *N*-acetylloline and *N*-formylloline, are produced by endophyte-infected tall fescue and have high insecticidal activity (Siegel and Bush, 1996; Dahlman et al., 1997). Furthermore, it appears that the fungus within the symbiotic relationship plays an important role in the synthesis of loline alkaloids (Wilkinson et al., 1997). Ultimately, it is the symbiosis between *Epichloë coenophiala* and tall fescue that allow this forage to thrive in the lower Midwest and Southern regions of the United States.

1.2.2 Ergot Alkaloids

Alkaloids are nitrogen containing organic compounds derived from plants and there have been approximately 10,000 alkaloids identified (Hesse, 1981). These alkaloids can be divided into five groups based on their chemical structures: heterocyclic alkaloids (most common and used for medicinal purposes), alkaloids with an exocyclic nitrogen (hallucinogenic), polyamine alkaloids, peptide alkaloids (ergot alkaloids), and terpene alkaloids. Previously mentioned loline alkaloids belong to the heterocyclic alkaloid group and have a close resemblance to amino sugars (Koskinen, 2012).

Ergot alkaloids were first identified in *Claviceps*, a parasitic fungus that infects many grasses and grains. However, it was soon found to be produced by a variety of fungi, including *Neotyphodium* and *Epichloë*, which has been previously described as the tall fescue endophyte (Jakubczyk et al., 2014). Ergot alkaloids belong to the peptide alkaloid group and there are three subclasses: clavines, lysergic acid amides, and ergopeptines (Young et al., 2015). There have been approximately 35 clavine alkaloids identified, however none used medicinally (Schiff, 2006). Lysergic acid amides are tetracyclic compounds and are highly active pharmacologically (Schiff, 2006; Young et al., 2015). Lysergic acid diethylamide (LSD) is a semisynthetic alkaloid derived from D-lysergic acid and is the most potent hallucinogen known (Young et al., 2015). The ergopeptines are a more complex amide derivative of D-lysergic acid. They are tetrapeptides containing lysergic acid and a combination of three amino acids which allows for great diversity among these alkaloids (Schiff, 2006; Guerre, 2015). Of the many ergopeptines, it has been identified that ergovaline is the most abundant and potent (Yates et al., 1985). The structure of ergovaline is D-lysergic acid with L-alanine, L-valine and L-proline and it accounts for approximately 90% of the total ergopeptines in tall fescue (Te Paske and Powell, 1993; Guerre, 2015).

1.2.3 Fescue Toxicosis

It was not long after the release of KY-31 tall fescue in 1943 when reports arose about animals performing poorly while grazing this forage (Cunningham, 1948; 1949). However, it was not until 1963 that the syndrome was named “fescue toxicosis” (Jacobson et al., 1963). Producers and researchers alike were perplexed why animals were performing so poorly while grazing this hardy forage. Bacon et al. (1977) was the first to suggest the fungal endophyte within the tall fescue plant as the likely culprit leading to the toxicity. Soon after, it was speculated that lysergic acid

amide derivatives could be toxic to animals (Porter et al., 1978). Finally, it was later confirmed that it was ergot alkaloids produced by the endophyte that cause fescue toxicosis (Lyons et al., 1986).

1.3 Physiological Effects of Fescue Toxicosis

As previously mentioned, approximately 35 million acres of land in the United States grow tall fescue (Burns and Chamblee, 1979; Strickland et al., 2009), and it has been estimated that 90% of these pastures are infected with the *Epichloë* endophyte (Siegel et al., 1985). Today, it is speculated that fescue toxicosis contributes to an approximate 8.62 million lbs. of beef lost per year and an estimated $1 to 2 billion in annual economic loss to the U.S. livestock industries (Strickland et al., 2011; Kallenbach, 2015). These losses can be attributed to the various symptoms associated with fescue toxicosis including decreased weight gain and feed intake, reduced prolactin secretion, vasoconstriction, retained winter hair coat, and hyperthermia (i.e. heat stress).

1.3.1 Reduced Average Daily Gains

After it was identified that the fungal endophyte was responsible for inducing fescue toxicosis (Bacon et al., 1977), numerous studies evaluated the impact of pastures having either high or low endophyte levels on daily gains by beef steers. High endophyte levels ranged from 60 to 100% and low endophyte levels ranged from 0 to 10% across many studies from several states. Collectively, the results indicated that steers grazing endophyte-infected tall fescue had decreased gains (Schmidt and Osborn, 1993). Specifically, a negative linear relationship exists between average daily gain (ADG) and endophyte level. This was first suggested by Williams et al. (1984) where data from a short-term grazing trial indicated that for every 10% increase in fungal infection level in a pasture this would result in an approximate decrease of 0.09 kg. A more extensive study was conducted by Crawford et al. (1989) where yearling steers and heifers grazed tall fescue

pastures over a three-year period. Endophyte infection levels ranged from 3 to 83% and ADG was described by the following equation:

$$\text{ADG (kg/day)} = 0.66 - (0.0045 \times \% \text{ endophyte})$$

Thus for every 10% increase in endophyte level, ADG would decrease by 0.045 kg/day (Crawford et al., 1989). Additionally, daily gains of steers fed tall fescue hay and seed from pastures that were either endophyte-free (EF) or endophyte-infected (EI) have been evaluated. Similarly to the grazing trials, the presence of the endophytic fungus reduced ADG in steers fed either the fescue hay diet (0.66 vs. 0.28 kg/day; EF vs. EI, respectively) or the fescue seed diet (0.96 vs. 0.20 kg/day; EF vs. EI, respectively; Schmidt et al., 1982). Recently, Liebe and White (2018) performed a comprehensive meta-analysis of existing literature reporting endophyte-infected tall fescue effects on ADG in cattle. Three measurements were tested: endophyte infection as a percentage of infected tillers (E%); ergovaline concentration in parts per billion [ppb] (E); and total ergot alkaloid concentration in ppb (TOTAL). It was found that for every 10% increase in E%, ADG would decrease by 0.038 kg/day (5.3%). Ergovaline concentrations ranged from 0 to 1,210 ppb and total ergot alkaloid concentrations ranged from 0 to 820 ppb with the models to describe the proportion of ADG loss as follows:

$$\text{ADG (kg/day)} = ([E] \times -3.254 \times 10^{-4}) + 0.7443$$

$$\text{ADG (kg/day)} = ([\text{TOTAL}] \times -3.934 \times 10^{-4}) + 0.5969$$

Where a 100 ppb increase in E or TOTAL was associated with a decrease in ADG of 0.033 kg/day (4.4%) and 0.039 kg/day (6.6%), respectively (Liebe and White, 2018).

Typically, endophyte-infected tall fescue grown during the autumn months is harvested as hay which then can be utilized during winter. Roberts et al. (2009) evaluated ergovaline concentrations throughout the 18 month storage period and observed that concentrations reduced

substantially, however they remained above 250 µg kg^{-1} dry matter (DM). This concentration exceeds the threshold level of 150 µg kg^{-1} DM that is believed to initiate symptoms associated with fescue toxicosis (Stamm et al., 1994). Additionally, the seed head contains the greatest concentrations of ergovaline ranging from 1,000 to 5,000 µg kg^{-1} DM during the summer months (Rottinghaus et al., 1991). Therefore, it is not surprising that ADG is reduced when steers are fed either endophyte-infected tall fescue hay or seed.

In addition to reduced weight gain experienced by animals consuming endophyte-infected tall fescue, feed intake is also reduced (Strickland et al., 1993). It has been suggested that the reduction in feed intake may in part explain the reduction in weight. However to eliminate the confounding effect of feed intake, Poole et al. (2018) designed a feeding regime to heifers that prevented changes in daily intake to examine the direct effects of ergot alkaloids on animal performance. While intake did not differ (18.5 vs. 18.1 kg/day; EF vs. EI, respectively), animal gains were reduced for heifers consuming ergot alkaloids (1.05 vs. 0.81 kg/day; EF vs. EI, respectively). It is speculated that elevated environmental temperatures (> 25°C) along with chronic exposure to ergot alkaloids, leads to other symptoms such as retained winter hair coat and vasoconstriction, which also contributed to the decrease in ADG during the study.

Finally, stockpiling tall fescue can be utilized during the winter months to provide a source of inexpensive grazing. Because tall fescue has a longer autumn growing season compared to other cool-season forages, it is an ideal forage to stockpile. Additionally, it has been shown that ergovaline concentrations decrease up to 85% during winter dormancy, thus suggesting that the forage becomes less toxic to grazing animals (Kallenbach et al., 2003). Furthermore, because of the reduced ergovaline concentrations in stockpiled endophyte-infected tall fescue, animal gains

are not reduced when compared to animals grazing stockpiled endophyte-free or novel endophyte fescue (0.51, 0.59, 0.56 kg/day, respectively; Drewnoski et al., 2009).

1.3.2 Hypoprolactinemia

A common symptom of fescue toxicosis is a suppression in prolactin secretion (hypoprolactinemia, Nasr and Pearson, 1975; Hurley et al., 1980). Prolactin is a polypeptide hormone consisting of 199 amino acids with three disulfide bonds between six cysteines (Cys^{4}-Cys^{11}, Cys^{58}-Cys^{174}, and Cys^{191}-Cys^{199}; Wallis, 1974). Secretion of prolactin is predominantly regulated by hypothalamic prolactin-inhibiting factors, specifically the monoamine neurotransmitter dopamine (Ben-Jonathan, 1985). Dopaminergic neurons originate in the arcuate nucleus (tonic center) of the hypothalamus and axon terminals of dopamine neurons are found in the median eminence (infundibulum) which are in close proximity to the primary capillaries of the hypophyseal portal venous plexus that funnel into the anterior pituitary (Kawano and Daikoku, 1987). This is known as the tuberoinfundibular dopaminergic system (TIDA; Holzbauer and Racke, 1985). Dopamine from the TIDA is considered the major regulator of prolactin secretion, and secretion is inhibited when dopamine is bound to its receptor on the lactotrophs of the anterior pituitary (Leong et al., 1983; Freeman et al., 2000). There are five isoforms of dopamine receptors that are divided into two subfamilies: D_1-like which is comprised of D_1 and D_5, and D_2-like which is comprised of D_2, D_3, and D_4 (Sibley and Monsma, 1992). On the pituitary lactotrophs, the dopamine receptors are primarily the D_2 dopamine receptor (Caron et al., 1978). The D_2 dopamine receptor is coupled to a $G_i\alpha$ protein which inhibits adenylyl cyclase activity, cyclic adenosine monophosphate (cAMP), and cytoplasmic calcium concentrations when dopamine or an agonist is bound (Enjalbert and Bockaert, 1983; Lledo et al., 1992), thus resulting in a suppression of prolactin secretion.

Prolactin concentrations have been shown to be reduced in animals consuming endophyte-infected tall fescue and this is often used as an indicator of fescue toxicosis (Strickland et al., 2011). It was observed that ergot alkaloids have structural similarities to numerous biogenic amines such as norepinephrine, serotonin, and dopamine (Berde, 1980), therefore it was suggested that ergot alkaloids may interact with pituitary dopamine receptors to enable an agonistic effect (Elsasser and Bolt, 1987). *In vitro* studies demonstrated that ergot alkaloids, specifically ergovaline, have a high affinity to the D_2 dopamine receptor and elicit agonistic effects that result in a decrease in prolactin secretion (Larson et al., 1995).

Prolactin secretion has also been linked with changes in environmental temperature. Schams and Reinhardt (1974) reported that prolactin concentrations were elevated during warmer verses cooler months in male and female calves. Similarly, Wettemann and Tucker (1974) observed that as ambient temperature incremented from 21 to 27°C prolactin concentrations increased linearly and as ambient temperature declined from 21 to 10°C prolactin concentrations decreased linearly. Interestingly, in two separate studies that evaluated the interaction between endophyte-infected tall fescue consumption (one study utilizing a fescue seed diet and the other utilizing a fescue hay diet) and environmental temperature (22 vs. 32°C) on physiological responses in cattle, both observed that prolactin concentrations were reduced for the endophyte-infected animals regardless of temperature effects (Boling et al., 1989; Aldrich et al., 1993). Furthermore, Aiken et al. (2013) evaluated the changes in prolactin concentrations in steers that previously grazed endophyte-infected tall fescue during the summer months (May to July). It was observed that prolactin concentrations after removal from endophyte-infected tall fescue increased and stabilized in approximately 15 days. Collectively, this demonstrates that as ambient temperature rises, cattle should experience an increase in prolactin concentrations; however if

cattle are grazing endophyte-infected tall fescue during this time then the agonistic effects of ergot alkaloids will inhibit prolactin secretion.

Finally, prolactin plays a critical role in mammary gland development and milk synthesis (Akers et al., 1981). It has been shown that cattle (beef and dairy) consuming endophyte-infected tall fescue display as much as 45 to 60% reduction in milk production (Schmidt and Osborn, 1993). Specifically, Strahan et al. (1987) demonstrated in lactating Holstein cows consuming endophyte-infected tall fescue a reduction in milk production compared to cows consuming endophyte-free or alfalfa-orchardgrass (15.6, 19.6, 21.3 kg/day, respectively). Additionally, forage intake was significantly reduced for the endophyte-infected cows versus the endophyte-free or alfalfa-orchardgrass cows (7.1, 9.1, 10.9 kg, respectively). In a similar study, Essig et al. (1993) observed reduced milk production per day in beef cows (Hereford and Angus) consuming endophyte-infected tall fescue compared to endophyte-free (1.25 vs. 3.65 kg). However, it is important to note that decreased prolactin concentrations does not directly impact milk yield (Walner et al., 1983), therefore it is speculated that other symptoms of fescue toxicosis, such as reduced feed intake or vasoconstriction, play a more critical role in the reduction of milk production.

1.3.3 Vasoconstriction

Aside from fescue toxicosis, fescue foot is another condition associated with consumption of KY-31. Fescue foot is a condition described as extreme lameness that could progress to peripheral necrosis of the foot, tail tip, and/or ear. Typically, fescue foot occurs during the cooler months and is less common in cattle that have access to tall fescue annually (Cunningham, 1949). Jensen et al. (1956) examined cattle post-mortem and concluded that the toxic compound, later identified as ergovaline, of tall fescue causes vasoconstriction and a reduction in blood flow to the extremities. Ergot alkaloid induced vasoconstriction is caused by thickening of the intimal layer

in the vascular and is a common symptom of both fescue foot and fescue toxicosis (Williams et al., 1975).

In order to simulate fescue toxicosis, early studies administered extracts of ergot alkaloids to cattle in order to elicit a short-term (acute) response and found that skin temperature decreased, indicating a reduction in blood flow to the extremities and an inability to properly dissipate body heat (Carr and Jacobson, 1969; Walls and Jacobson, 1970; Rhodes et al., 1991; McCollough et al., 1994; Al-Haidary et al., 1995; Browning Jr. et al., 1998a). Specifically, a couple of short-term studies have evaluated the effect ergovaline has on skin temperature. McCollough et al. (1994) intravenously injected calves with three ergot alkaloids (ergotamine, ergine, and ergovaline) and observed that ergovaline was a more effective vasoconstrictor and greatly reduced tail skin temperature compared to ergotamine and ergine. Additionally, Al-Haidary et al. (1995) injected cattle with ergovaline (5.2 µg/kg of BW/day) and found that skin temperature was reduced. Together, these short-term studies indicate that ergot alkaloids, specifically ergovaline, have vasoconstrictive properties that reduce blood flow to the extremities and alter thermoregulation.

The benefit to short-term response studies is that researchers can evaluate the effect ergot alkaloids on vasoconstriction within a few hours or days, however these studies may not provide a realistic image of animals suffering from fescue toxicosis. Therefore, few long-term (chronic) response studies have been conducted to evaluate the impact that consumption of ergot alkaloids has on vasoconstriction. Similar to the short-term response studies, cattle consuming endophyte-infected tall fescue experience peripheral vasoconstriction and a reduction skin temperature (Osborn, 1988; Rhodes et al., 1991), and ergovaline is the most potent vasoconstrictor (Al-Haidary et al., 2001). Ergovaline has also been shown numerous times *in vitro* to be an effective vasoconstrictor compared to lysergic acid and other ergot alkaloids (Klotz et al., 2007; 2008; 2009;

2010), and exposure to ergovaline can result in bioaccumulation within the vasculature, therefore prolonging the effects of ergot alkaloid induced vasoconstriction (Klotz et al., 2009). Additionally, a couple of studies have used Doppler ultrasonography to show that heifers chronically exposed to endophyte-infected tall fescue have reduced caudal artery area and blood flow to the peripheral arteries when compared to heifers consuming endophyte-free tall fescue (Aiken et al., 2007; Poole et al., 2018).

Ergot alkaloids induce a vasoconstrictive response by interacting with biogenic amine receptors including serotonergic and adrenergic receptors (Pertz and Eich, 1999). Serotonin (5-hydroxtryptamine; 5-HT) receptors are located in the central and peripheral nervous systems and smooth muscle throughout the body and consist of seven structurally different classes (at least 14 subtypes), six of which are G-protein coupled receptors and one (5-HT_3) is a ligand-gated ion channel (Barnes and Sharp, 1999). Dyer (1993) was the first to report the interaction between ergovaline and 5-HT receptors. Ergovaline elicited a vasoconstrictive response via 5-HT_2 receptor in both bovine uterine and umbilical arteries. Moreover, it has been repeatedly demonstrated *in vitro* utilizing a bovine lateral saphenous vein bioassay that the 5-HT_{2A} receptor subtype induces vasoconstriction via exposure to ergot alkaloids, specifically ergovaline and ergotamine (Klotz et al., 2012; 2013). Additionally, steers continuously exposed to endophyte-infected tall fescue seed have suppressed gene expression of the *5-HT_{2A}* receptor subtype in the smooth muscle of the foregut, small intestine, large intestine, and cecum (Klotz et al., 2014). The 5-HT_{2A} receptor subtype is associated with smooth muscle contraction and the authors speculated that continuous exposure to ergot alkaloids results in a downregulation of the 5-HT_{2A} receptor and may alter gut motility.

As previously mentioned, ergot alkaloids interact with other biogenic amine receptors, specifically α-adrenergic receptors to induce a vasoconstrictive response. The α-adrenergic receptors are G-protein coupled receptors and divided into two subfamilies: α_1 and α_2, where stimulation of both results in smooth muscle contractions (Strosberg, 1993). It has been reported that α_1 receptors are primarily located in arterial blood vessels while α_2 receptors in venous blood vessels (Oliver, 1997), and ergot alkaloids have a greater affinity towards α_2-adrenergic receptors (McPherson and Beart, 1983; Oliver et al., 1998). Aiken et al. (2013) evaluated the length of time required to alleviate vasoconstriction in steers that previously grazed endophyte-infected tall fescue during the summer months (May to July). After removal from endophyte-infected tall fescue, it was observed via color Doppler ultrasonography that vasoconstriction of the caudal artery may take more than 30 days to stabilize. Furthermore, an additional study found that recovery occurs after 36 days off endophyte-infected pasture and while adrenergic receptors were affected, 5-HT_{2A} receptors appear to be more dramatically affected (contractile response [% response induced by 1×10^{-4} M norepinephrine]) by ergot alkaloids (Klotz et al., 2016).

1.3.4 Rough Hair Coat

An early recognized and well-established symptom exhibited by cattle experiencing fescue toxicosis is a rough hair coat (winter coat retention during the summer months; Cunningham, 1949; Yates, 1962). It has been recognized that performance of cattle is correlated with coat type; and that a rough hair coat acts as an insulator that affects efficiency of evaporative cooling (i.e. reduction in temperature as a result of evaporation of a liquid) and thermoregulatory properties (i.e. skin and rectal temperature, and respiration rate; Turner and Schleger, 1960; Turner, 1962).

There are numerous theories as to why cattle grazing endophyte-infected tall fescue display a rough hair coat during the summer months. As previously mentioned, prolactin concentrations

have been associated with changes in environmental temperature (i.e. photoperiod), with prolactin increasing during warmer months (increased day length) and decreasing during cooler months (decreased day length; Schams and Reinhardt, 1974; Wettemann and Tucker, 1974). Porter and Thompson (1992) speculated that the decrease in prolactin observed during cooler months is responsible for winter hair growth, and likewise the increase in prolactin observed during warmer months triggers hair shedding. Notability, prolactin has been shown to play a role in hair shedding and growth in other mammalian species, with prolactin receptors being located in mouse hair follicles (Foitzik et al., 2003) and sheep wool follicles (Nixon et al., 2002). It has been speculated that fescue toxicosis induced hypoprolactinemia prevents shedding of the winter hair coat, therefore cattle have an elevated body temperature and increased vulnerability to heat stress (Aiken et al., 2011).

Given the variation in coat type between groups of cattle (*Bos taurus* vs. *Bos indicus*), few studies have adapted a simple scoring system for hair coat types/shedding and estimated the heritability of this trait. Turner and Schleger (1960) utilized approximately 500 Hereford (*Bos taurus*) and Shorthorn (*Bos taurus*) cows and their progeny, which belonged to eight breed groups resulting from mating the two breeds of cows with four breeds of bulls including Hereford *(Bos taurus*), Shorthorn (*Bos taurus*), Africander (*Bos indicus*), and Brahman (*Bos indicus*). The scoring system is as follows:

Coat Score (1-7)	Coat Type	Brief Description
1	Extremely short	Hairs extremely short, found in Zebu cows.
2	Very short	Coat sleek, just able to be lifted by thumb
3	Fairly short	General appearance smooth-coated
4	Fairly long	Coat not completely smooth, patches of hairs
5	Long	Hairs distinctly long; predominantly coarse
6	Woolly	Hairs erect, giving fur-like appearance
7	Very woolly	Greater length, heavy cover extending to neck and rump

Adapted from Turner and Schleger, 1960.

It was estimated that the heritability (h^2) of coat scores was 0.63, and that there was a moderate genetic correlation between hair coat type and calf weaning weight (r^2 = -0.503). A similar study evaluated the utilization of a slightly different scoring system in order to estimate the h^2 of hair coat shedding and the relationship between hair coat shedding and calf weaning weight in Purebred Angus cattle. The scoring system is as follows:

Hair Shedding Score	Description
1	Slick, short summer coat
2	Coat is mostly shed
3	Coat is halfway shed
4	Coat exhibits initial shedding
5	Full winter coat

Adapted from Gray et al., 2011.

It was found that h^2 of hair shedding scores was 0.35, and similar to Turner and Schleger (1960) a moderate genetic correlation with calf weaning weight (r^2 = -0.58; Gray et al., 2011). Interestingly, these cattle were located in the piedmont of North Carolina and the results indicate that even while cattle were grazing endophyte-infected tall fescue there was still variation in hair coat shedding scores within the herd. Thus, suggesting that some animals are more adapted to this environment and still productive while grazing endophyte-infected tall fescue.

1.3.5 Heat Stress

Heat stress can be defined as an environment acting to elevate normal body temperature and can disrupt physiology and performance in an animal (Fuquary, 1981). In a normal animal, body temperature is characterized as remaining within ± 1°C of normal body temperature (Guyton, 1986), and the maintenance of normal body temperature is critical for proper function of thermoregulatory and metabolic processes. The combination of previously described symptoms of fescue toxicosis including hypoprolactinemia, vasoconstriction, and a rough hair coat ultimately results in altered thermoregulatory properties and may result in an animal suffering from heat stress

related symptoms. These symptoms include, but are not limited to, elevated rectal temperatures, high respiration rates, reduced heart rates, and altered animal behavior in which animals seek shade and/or water and consequently spend less time grazing (Schmidt and Osborn, 1993). In fact, symptoms associated with heat stress and fescue toxicosis are often confounded and difficult to separate. However, Osborn (1988) demonstrated interesting interactions in steers consuming either endophyte-free (E-) or endophyte-infected (E+) diets in a 21°C (thermoneutral) or 32°C (heat stress) environment. In the thermoneutral environment, steers on the E+ diet had reduced daily feed intake (4.3 vs. 6.7 kg) and heart rate (53 vs. 70 beats per minute) indicating signs of fescue toxicosis, however rectal temperature and respiration rate did not differ. Interestingly in the heat stress environment, steers on the E+ diet had reduced daily feed intake (2.2 vs. 5.4 kg), reduced heart rate (49 vs. 65 beats per minute), elevated rectal temperature (40.4 vs. 39.9 °C), and respiration rate (95 vs 78 breaths per minute) when compared to the E- diet. Thus, demonstrating that when ambient temperature is high signs and repercussions of fescue toxicosis are amplified.

The thermoregulatory center is located within the hypothalamus and onset of heat stress will trigger the hypothalamus to induce a vasodilatory response to the peripheral tissues in order to dissipate excess body heat (Curtis, 1983). It has been estimated that approximately 85% of metabolic heat produced by cattle is transported to the skin for heat dissipation and evaporative cooling (i.e. sweating) while the remaining 15% is lost via the respiratory tract (i.e. panting; Finch, 1986). Unfortunately, consumption of ergot alkaloids induces vasoconstriction to the periphery and negatively impact normal thermoregulatory methods to resolve heat stress and ultimately does not allow an animal to properly dissipate body heat. McClanahan et al. (2008) demonstrated that the endophyte-infected tall fescue induced peripheral vasoconstriction causes sweat production to decline in grazing animals. This indicates that cattle suffering from fescue toxicosis are unable to

dissipate body heat via evaporative cooling and are completely reliant on panting and other means (i.e. seeking shade and/or water) to reduce body temperature.

1.3.6 Feedlot Performance and Carcass Traits

As previously mentioned, cattle grazing endophyte-infected tall fescue experience reduced growth and this has been attributed to a decrease in nutrient intake (Paterson et al., 1995). This reduction in growth performance is most detrimental to stocker steers, as this is the time for substantial weight gain before feedlot entry (Herring, 2014). A few studies have evaluated feedlot performance of steers that previously grazed endophyte-infected tall fescue during the stocker phase. Most researchers observed compensatory gain in the feedlot (Cole et al., 1987; Lusby et al., 1990; Beconi et al., 1995). Notably, Lusby et al. (1990) reported feedlot performance and carcass characteristics in stocker steers (12 to 19 months of age) that grazed either high (HE) or low (LE) endophyte-infected tall fescue pastures from November to May in eastern Oklahoma. Upon feedlot arrival in western Oklahoma, HE steers had reduced body weight, however there was no difference in rectal temperatures between groups. After the 112d finishing phase, HE steers experienced compensatory gain of up to 67% of the reduced gains (e.g. after the grazing period HE steers weighed approximately 46 kg less than LE steers, but gained about 31 kg more during the finishing phase), however still had reduced carcass weights and carcass quality grade (Lusby et al., 1990). Contrary to this, similar studies failed to detect compensatory gain (Duckett et al., 2001; Scaglia et al., 2013). Specifically, Duckett et al. (2001) reported feedlot performance and carcass characteristics in stocker steers (~295 kg) that grazed non-toxic endophyte-infected tall fescue (NT), endophyte-free tall fescue (EF), or toxic endophyte-infected tall fescue (EI) for 63d in central Georgia. Upon feedlot arrival in Oklahoma, EI steers had reduced body weight and elevated rectal temperatures compared to NT and EF steers. After the 112d finishing phase, there was no

difference in ADG or carcass characteristics between groups (Duckett et al., 2001). The inconsistency between the findings of Lusby et al. (1990) and Duckett et al. (2001) could be explained by environmental factors, such as ambient temperature and relative humidity, time of year when cattle were placed in the feedlot, and/or the extreme difference in travel distance to the feedlot.

Coffey et al. (1992) evaluated two types of management strategies to mitigate fescue toxicosis symptoms during the summer months on stocker steer growth and subsequent feedlot performance. The two management strategies include continuously grazing on endophyte-infected tall fescue overseeded with ladino clover (T) or steers were rotated to a bermudagrass pasture (B). During the stocker phase, initial BW as similar between groups, however T steers had a greater ADG and final BW compared to B steers. During the feedlot phase, B steers experienced some compensatory gain, but T steers had greater HCW and dressing percentage (% = (carcass weight/live weight) x 100) at harvest. The differences observed between the two management strategies most likely could be explained by the stocking densities in which there were 2.5 steers/hectare (ha) on the T pastures versus 6 steers/ha on the B pastures. Ultimately, the results suggest that there are management strategies available to stocker operations that want to graze tall fescue pastures, yet have minimal negative impact on stocker and feedlot performance.

A limited number of studies have been able to quantify the accumulation of ergot alkaloids in animal tissues (i.e. muscle, fat). Realini et al. (2005) evaluated carcass traits, meat quality, and fatty acid profiles in Hereford steers (273 ± 35 kg) finished on endophyte-infected (EI) or novel (N) tall fescue for 176d in central Georgia. Average daily gain and HCW were reduced in EI steers, however there were no differences in other carcass traits. Adipose tissue from EI steers contained a greater percentage of saturated fatty acids and reduced percentage of monounsaturated fatty

acids, with subcutaneous fat having greater total ergot alkaloid (those that contain the ergoline ring structure) concentrations. However, these data did not specifically demonstrate an accumulation of ergovaline. Moreover, the bioaccumulation and metabolism of ergot alkaloids in beef cattle is not well defined and the impact it has on food safety for the consumer, remains unknown.

1.4 Female Reproduction in Cattle

1.4.1 Estrous Cycle Pituitary, Ovarian, and Uterine Function

Cattle are classified as non-seasonal polyestrous, which means having a uniform distribution of estrous cycles throughout the year. The estrous cycle in cattle is generally 21 days, but can range from 17 to 24 days in length. There are two distinct phases of the estrous cycle: the luteal phase (14-18 days) and the follicular phase (4-6 days). The luteal phase is characterized as the period when the corpus luteum (CL) forms post-ovulation and the follicular phase is the period following regression of the CL (luteolysis) to ovulation (Hansel and Convey, 1983). The processes of ovulation, formation of the CL, and luteolysis are all essential for proper cyclcity, and these dynamics require coordination between the anterior pituitary, ovary, and uterus.

Primordial follicles are established in the ovary during fetal development and form the basis of all subsequent follicular growth in adulthood. Follicle growth and development (i.e. folliculogenesis) begins with primordial follicles receiving signals from the anterior pituitary: the gonadotropins, follicle stimulating hormone (FSH) and luteinizing hormone (LH). Follicle stimulating hormone initiates the proliferation of granulosa cells and aid in the transition from primordial to primary and secondary follicles. In cattle, tertiary or antral follicle growth occurs in 2 to 3 waves per cycle, with each wave having recruitment, selection, and dominance of follicles that will either result in atresia or ovulation (Rajakoski, 1960; Fortune et al, 1988; Ginther et al., 1989). On average, follicle stimulating hormone peaks on days 2, 9, and 16 of the estrous cycle

and this coincides with the emergence of recruited follicles (Bryner et al., 1990; Adams et al., 1992). These follicles are FSH-dependent with FSH receptors located on the granulosa cells. As these follicles grow and undergo selection, granulosa cells become more responsive to LH and theca cells will produce androgens which are converted primarily to estradiol (E_2) in the granulosa cells (Fortune and Quirk, 1988; Richards, 1994). As E_2 and inhibin concentrations produced by the growing selected follicles increase, FSH production by the anterior pituitary is suppressed via negative feedback (Law et al; 1990; Turzillo and Fortune, 1990). A few selected follicles will continue to grow into dominance and produce more E_2, while others will become atretic. During the final follicular wave (post-luteolysis), large concentrations of E_2 produced by the preovulatory follicle will act via positive feedback to trigger the preoptic nucleus of the hypothalamus to release a surge of gonadotropin releasing hormone (GnRH), which will stimulate the anterior pituitary to release the preovulatory surge of LH and ovulation will occur (Wettemann et al., 1972; Kinder et al., 1983; Day et al., 1986).

When a follicle ovulates, the granulosa and theca cells undergo dramatic changes into luteal cells, a process known as luteinization. This transformation is governed by LH and locally produced prostaglandin E_2 (PGE_2) will induce hyperemia and vasodilation which aids in the luteinization process by facilitating the supply of LH to the ovary (Kaltenbach et al., 1968; Murdoch et al., 1981; Farin et al., 1988). Specifically during this transformation, granulosa cells will differentiate into large luteal cells, while theca cells differentiate into small luteal cells (Alila and Hansel, 1984; O'Shea et al., 1987). Farin et al. (1986) reported that large luteal cells will increase in size (i.e. hypertrophy) while small luteal cells will increase in number (i.e. hyperplasia) during luteal development. An additional and essential component to CL formation is development of new blood vessels, known as angiogenesis. There are numerous angiogenic factors, but perhaps

the two most important are fibroblast growth factors (FGFs) and vascular endothelial growth factors (VEGFs). Specifically, FGF-2 (also known as basic FGF [bFGF]) is believed to be the predominant FGF responsible for angiogenesis, since mRNA expression is highest during the early luteal phase (Schams et al., 1994). Similarly, VEGF mRNA expression was highest during the early luteal phase which coincides with luteal vascularization (Berisha et al., 2000). It is believed that the functionality of the CL (i.e. production of progesterone [P_4]) is dependent on the degree of vascularization (Reynolds et al., 2000; Stocco et al., 2007). Due to the luteinization process, the original steroidogenic pathway in preovulatory follicles switches from E_2 to P_4, the primary steroid hormone produced by the CL. It has been demonstrated that LH, not prolactin, is the predominant luteotropic hormone responsible for maintenance of the CL and production of P_4 (Kaltenbach et al., 1968; Hoffmann et al., 1974; Farin et al., 1988). Approximately 17 days post-ovulation, if fertilization does not occur then the CL will undergo regression (Shemesh et al., 1968; Northey and French, 1980). Wiltbank and Casida (1956) were the first to demonstrate in cattle the involvement of the uterus in luteolysis by preforming hysterectomies and observing maintenance of the CL on the ovary. About 15 years later, prostaglandin $F_2\alpha$ ($PGF_2\alpha$) was identified as the uterine luteolytic factor responsible for the regression of the CL (Rowson et al., 1972; Louis et al., 1974).

1.4.2 Oocyte and Early Embryonic Development

The mammalian oocyte originates from a small number of stem cells called primordial germ cells (PGCs) within the prenatal ovary (Eddy et al., 1981), this is the beginning of oogenesis. The PGCs will begin to migrate into the ovarian cortex and PGCs will mitotically divide into oogonia. The final mitotic division of the oogonia to primary oocyte is extremely critical because it is the final DNA replication to occur before the oocyte enters the first meiosis (meiosis I) and

initiates meiotic maturation. The first phase of meiosis I is prophase I and this phase can be further divided into five phases based on chromosome structure: leptotene, zygotene, pachytene, diplotene, and diakinesis. When the primary oocyte develops to the diplotene phase, the oocyte is then arrested and this is referred to as the dictyate stage (germinal vesicle [GV] stage; Baker and Franchi, 1967; Downs, 1993). The oocyte will reach the dictyate stage before or shortly after birth and remain arrested at this stage until resumption of meiosis occurs at/after puberty (Masui et al., 1985).

As an oocyte develops to the dictyate stage, they will quickly become associated with pregranulosa cells (together forming primordial follicles) which are believed to initiate the signal that results in meiotic arrest (McLaren, 1988). It is known that one of the factor(s) associated with the maintenance of meiotic arrest is cAMP (Sirard and Bilodeau, 1990; Aktas et al., 1995). During the dictyate stage, the oocyte will undergo significant growth as ovarian follicles progress through the primary and secondary stages of folliculogenesis. In fact, the oocyte will increase in diameter approximately three-fold prior to the formation of antrum within tertiary (antral) follicles (Wassarman and Albertini, 1994). In cattle, after the formation of the antrum, and once the oocyte has reached a diameter of approximately 100 to 110 μm, the LH surge will initiate resumption of meiosis (Eppig, 1991; Fair et al., 1995). The continuation of meiotic maturation encompasses both nuclear and cytoplasmic events (maturation) which occur simultaneously and will result in a developmentally competent oocyte. Nuclear maturation begins with chromatin condensation and nuclear (germinal vesicle [GV]) breakdown (GVBD) which occurs approximately 4-8 hours post-LH surge (Kruip et al., 1983). Following GVDB, maturation- (or metaphase-) promoting factor (MPF) will steadily increase during metaphase I then sharply decrease during anaphase I (Hampl and Eppig, 1995). Because of the unique organization of the meiotic spindle in mammalian

oocytes, this results in an unequal division of cytoplasm (telophase I) and subsequent extrusion of the first polar body into the perivitelline space (i.e. space between the oocyte cytoplasm and zona pellucida; Albertini et al., 1993). The now secondary oocyte will begin meiosis II and become arrested at metaphase II (MII), which is sustained by high levels of MPF until fertilization occurs. As previously stated, while the oocyte is undergoing nuclear maturation, cytoplasmic maturation is simultaneously occurring and is necessary to prepare for fertilization and embryonic development. Cytoplasmic maturation includes structural and molecular changes occurring within organelles of the oocyte cytoplasm. For example, the endoplasmic reticulum membranes are responsible for the establishment of calcium (Ca^{2+}) storage and release which plays an important role in intracellular signaling (Koch, 1990). The release of Ca^{2+} is mediated by inositol 1, 4, 5 triphosphate (IP_3) binding to its receptor on the endoplasmic reticulum membrane, and this is essential for oocyte activation during fertilization (Kline and Kline, 1994). Interestingly, when oocytes are microinjected with IP_3 during early stages of meiotic maturation, Ca^{2+} release is low, however when oocytes reach MII, IP_3-R reach maximum sensitivity to IP_3 and Ca^{2+} release is high (Fujiwara et al., 1993). Therefore, due to cytoplasmic maturation the IP_3-R on the endoplasmic reticulum membranes build sensitivity to IP_3 and allow for release of Ca^{2+} when fertilization occurs.

In cattle, post-LH surge it takes approximately 18-24 hours for an oocyte to progress to MII (Kruip et al., 1983). Meiosis II will only resume if fertilization occurs. After ovulation, the oocyte and surrounding cumulus cells (cumulus oocyte complex, COC) will enter the oviduct via the ciliated fimbria of the infundibulum and transport through the ampulla to the ampullary-isthmic junction where fertilization occurs (El-Banna and Hafez, 1970). Sperm that have undergone capacitation and hyperactivity are able to bind to the zona pellucida, specifically binding to a

glycoprotein called zona protein 3 (ZP3) which will induce the acrosome reaction. The acrosome reaction will allow the sperm to penetrate through the zona pellucida and upon reaching the perivitelline space will fuse with the oocyte plasma membrane (i.e. vitelline membrane; Crozet, 1984). Following fusion, the oocyte will undergo the cortical reaction in which cortical granules will be subjected to exocytosis and their contents released into the perivitelline space, this results in the zona pellucida "hardening" so that other sperm cannot penetrate and thus preventing polyspermy (Wassarman, 1992). As the secondary oocyte resumes meiosis II, extrudes the second polar body, and becomes haploid, the male pronucleus (haploid, 1N) will become larger in size and begin to migrate towards the center of the cytoplasm meeting the female pronucleus. Following fusion of the pronuclei (i.e. syngamy), the one-cell embryo is now referred to as a zygote (diploid, 2N) and will undergo a series of mitotic (cleavage) divisions (Schultz and Kopf, 1995).

In cattle, the progression to the first cell cleavage occurs approximately 23-30 hours post-fertilization. Cleavage to the 4-cell stage occurs approximately 36-50 hours after fertilization, and the 8-cell stage is reached at 56-64 hours (Hamilton and Laing, 1946; Hyttel et al., 1988; Barnes and Eyestone, 1990). At the 8-cell stage, the maternal genome which has been controlling all aspects of embryonic development, becomes inactivated and the embryonic genome is activated; this is known as maternal to zygotic (embryonic) transition (MZT or MET). Therefore, development beyond the 8-cell stage is solely dependent on embryonic genome transcriptional activity (Barnes and Eyestone, 1990; Telfored et al., 1990), and this transition is extremely important to embryonic survival, as it allows the embryo to respond to environmental stresses. After MET has occurred, the embryo cells (i.e. blastomeres) will begin to compact and this is referred to as the morula stage. The morula consists of internal and external blastomeres that will eventually form the cell types of the blastocyst (inner cell mass [ICM] and trophoblast cells,

respectively). Initially, the morula does not contain an internal cavity, however, the external cells will begin to secrete sodium into the intracellular space and fluid will begin to accumulate forming an internal cavity, otherwise known as the blastocoel cavity (Gilbert, 2000). Once a distinct cavity has formed, the embryo is referred to as a blastocyst and this stage of development is generally observed 7 days post-fertilization in cattle.

In vitro production of bovine embryos has been used as a research tool for decades. The process first involves oocyte retrieval from either transvaginal ovum pick-up or aspiration of abattoir collected ovaries. Harvested COCs (oocyte and cumulus granulosa cells) are then subjected to *in vitro* maturation (IVM). Typically for IVM, many laboratories utilize tissue culture medium (TCM) 199 supplemented with 10% estrus cow serum or fetal calf serum, E_2, FSH, and LH at 5% CO_2 at 35.8°C for 20-24 hours (Galli and Lazzari, 1996). It is of critical importance that the surrounding cumulus cells are present due to their vital role in cytoplasmic maturation and subsequent development (Konishi et al., 1996). Preparation of sperm for *in vitro* fertilization (IVF) is then accomplished by either a Percoll density gradient with centrifugation or a swim-up procedure. Additionally, sperm must undergo capacitation which is done by the inclusion of hyaluronic acid or heparin in the IVF medium (Parrish et al., 1986; Eppig, 1996). Capacitated sperm and COCs are co-incubated for 18 to 20 hours and following co-culture, the cumulus cells are removed (Galli and Lazzari, 1996). Finally, presumptive zygotes undergo *in vitro* culture (IVC) for approximately 168-192 hours (6-7 days) until the blastocyst stage. The IVC medium range from undefined medium such as TCM199 supplemented with serum to a fully defined medium such as synthetic oviductal fluid (SOF) in which all components are known in the medium (Galli and Lazzari, 1996).

1.4.3 Maternal Recognition of Pregnancy

During early gestation, it is essential that the maternal system recognize the presence of a growing conceptus (embryo and its extra-embryonic membranes) within the uterus, and prevents luteolysis to prolong the lifespan of the CL and P_4 production (= maternal recognition of pregnancy [MRP]). Interferon-tau (IFN-τ) is the factor responsible for MRP in ruminants. Produced by the trophoblast cells, IFN-τ is a member of the Type 1 INF family and plays a role in immune response after viral infection and possess anti-proliferative properties (Friedman, 1981).

Expression of IFN-τ can first be detected at low levels during the morula to early blastocyst stage (day 6-7 of development), however steadily increase until it peaks around day 16 of pregnancy (around the time of MRP; Kubisch et al., 1998). In a non-pregnant ruminant, oxytocin (OT) acts on localized receptors (OT-R) on the endometrium to stimulate synthesis of $PGF_2\alpha$, thus triggering luteolysis (Wathes and Lamming, 1995; McCracken et al., 1999). Interferon-tau will prevent luteolysis in a pregnant ruminant by inhibiting estrogen receptor expression and E_2-induced expression of OT-R in the uterine epithelium (Thatcher et al., 1989), thus OT cannot induce $PGF_2\alpha$ secretion. The expression of IFN-τ by the trophoblast cells is short-lived and dramatically decreases around day 19 to 21 in cattle, corresponding with uterine attachment (Ealy et al., 2001).

Both radio- or enzyme-linked immunoassays (ELISA) have been developed for quantifying IFN-τ (Zhu et al., 1996; Takahashi et al., 2005), however these assays have not been commercialized. Therefore, cattle producers are unable to utilize these products as a method to confirm pregnancy. Luckily, there is an easy, alternative ELISA commercially available for producers that detects pregnancy-specific protein B (PSPB; Sasser et al., 1986). Specific to ruminants, this is a glycoprotein that is produced by the placenta, specifically the bi-nucleated cells

which form on approximately day 21 of gestation. In cattle, PSPB levels are high enough 28 days post-breeding to be used to classify animals as pregnant (Patel et al., 1995).

1.5 Ergot Alkaloids and Reproduction

The overwhelming majority of small-scale cow-calf operations are located in the central and eastern portion of the U.S. and predominantly graze tall fescue (Schmidt and Osborn, 1993). As a result, these cattle typically succumb to fescue toxicosis and have impaired reproductive function which negatively impacts the productivity and profitability of a cow-calf operation. Reproductive failure can be attributed to altered ovarian follicle development, luteal dysfunction, and reduced circulating steroid hormone concentrations which all which contribute to reduced pregnancy rates experienced by cattle suffering from fescue toxicosis (Porter and Thompson, 1992).

1.5.1 Ergot Alkaloids and Folliculogenesis

There is evidence that the consumption of ergot alkaloids will impair folliculogenesis. McKenzie and Erickson (1989, 1991) observed a decrease in the diameter and number of large follicles in heifers consuming endophyte-infected tall fescue. Likewise, Burke and Rorie (2002) examined follicular development and function in lactating beef cows grazing endophyte-free (EF) or endophyte-infected (EI) tall fescue. No differences in the number of class 1 (small; 3 to 5 mm) and class 3 (large; >10 mm) antral follicles between treatments were observed. Conversely, the number of class 2 (medium; 6-9 mm) follicles were reduced in cows grazing EI fescue compared to cows grazing EF fescue. However, since fescue toxicosis symptoms, such as reduced gains and heat stress, have been previously shown to impair folliculogenesis (Murphy et al., 1991; Roth et al. 2001), it is difficult to determine whether the observed effects of fescue toxicosis on follicle development are direct or indirect from these studies. Therefore, Burke et al. (2001) investigated

the interaction between heat stress and consumption of endophyte-infected fescue on follicular dynamics. Dietary intake for the heat stressed heifers on both diets was similar to minimize these confounding effects. It was observed that the combination of heifers consuming the endophyte-infected seed togetherwith heat stress conditions resulted in a smaller preovulatory follicle diameter and that consumption of the seed alone (without heat stress) also resulted in a decrease in the number of large follicles (> 9 mm).

With vasoconstriction being a major symptom of fescue toxicosis, Poole et al. (2018) wanted to investigate if chronic exposure of ergot alkaloids would reduce systemic blood flow to the ovary during various stages of the estrous cycle, and thus may hinder follicular development. Ovarian artery and vein area was measured via Doppler ultrasonography on days 0, 4, 10, and 17 to represent both the follicular and luteal phases of the estrous cycle. It was observed that ovarian artery area was not different on days 0 and 4, however did differ on days 10 and 17 with heifers consuming ergot alkaloids. Additionally, ovarian vein area was reduced in heifers consuming ergot alkaloids on days 0 and 17. In regards to follicular development, it was observed that 6 to 9 mm follicle number was reduced in heifers consuming ergot alkaloids. The 6 to 9 mm follicle size can be classified as selected follicles, and are of critical importance to follicular development with the gonadotropin dependence switching from FSH to LH. These results suggest that exposure to ergot alkaloids may hinder follicular selection though inadequate delivery of gonadotropins and other nutrients due to insufficient blood flow to the ovary.

In addition to impairment of folliculogenesis, exposure to ergot alkaloids alter reproductive hormone concentrations that govern follicular development. Browning Jr. et al. (1997) first demonstrated in steers receiving injections of ergotamine tartrate that LH concentrations were reduced. Subsequently, these investigators determined whether acute ergot alkaloid exposure

would alter LH and FSH in cows during the late luteal phase (day 15 or 16 post-estrus). It was observed that LH concentrations were reduced 4 hours post injection, however FSH concentrations did not differ between the cows receiving ergotamine tartrate and the saline control (Browning Jr. et al., 1998b). Unfortunately, the authors did not evaluate follicle dynamics or E_2 concentrations, however this may be due to this being an acute response study. A few studies have evaluated the impact of ergot alkaloids on E_2 secretion. As previously mentioned, Burke et al. (2001) evaluated the interaction between heat stress and consumption of endophyte-infected fescue on follicular dynamics and E_2 secretion. In the thermoneutral environment, E_2 concentrations were reduced in heifers consuming endophyte-infected fescue compared to the control heifers. Additionally, heat stress conditions reduced E_2 concentrations regardless of treatment diet. Interestingly, a follow-up study by Burke and Rorie (2002) did not see in difference in E_2 concentrations between postpartum cows consuming either endophyte-free or -infected tall fescue. Together, these studies demonstrate that ergot alkaloids alone can impair follicular development and E_2 secretion perhaps due to reduced blood flow to the ovary, and that heat stress conditions exacerbate these negative effects.

1.5.2 Ergot Alkaloids and Corpus Luteum Function

Reduced pregnancy rates is a common symptom experienced by cattle suffering from fescue toxicosis. In order for pregnancy to be maintained in cattle, luteolysis must be prevented and P_4 concentrations sustained. With this in mind, many researchers speculated that ergot alkaloid exposure would result in luteal dysfunction, thus reducing pregnancy rates. Estienne et al. (1990) observed altered luteal function in heifers on endophyte-infected fescue by showing that heifers which had a CL present via ultrasonography, displayed a reduction in circulating P_4 concentrations. Interestingly, a separate study found that even if heifers appeared to be cycling and ovulating normally, there were cellular changes (fewer nuclei and a greater number of large luteal cells with

increased diameter) of the CL in heifers grazing endophyte-infected fescue which may contribute to altered functionality (Ahmed et al., 1990). Mahmood et al. (1994) examined luteal function in heifers grazing either low (0%) or high (>75%) endophyte-infected pastures for 168 days. Heifers were synchronized with $PGF_2\alpha$ on days 101 and 112 of the trial and P_4 concentrations were determined on days 112, 116, 120, and 124. Heifers on the high endophyte-infected pastures had either low P_4 concentrations (< 1.5 ng/ml) after synchronization or relatively high P_4 concentrations (> 1.5 ng/ml) that would sharply decrease, thus indicating either short luteal phases (lifespan of the CL is often 10 days or less; Odde et al., 1980) or luteal dysfunction.

One theory to explain the endophyte-infected fescue induced luteal dysfunction is a reduction in the steroid hormone precursor, cholesterol. Produced in the liver, cholesterol is derived from low-density and high density lipoprotein then transported and utilized by the bovine CL (Pate and Condon, 1982; 1989). A few studies have shown that cholesterol concentrations were decreased in cattle consuming endophyte-infected tall fescue (Rice et al., 1997; Burke et al., 2001; Nihsen et al., 2004). Moreover, Burke et al. (2001) observed that heat stress conditions further reduced cholesterol concentrations in heifers consuming endophyte-infected fescue. Likewise in the same study, progesterone concentrations were reduced to a greater extent in heifers consuming endophyte-infected fescue subjected to heat stress conditions.

Another less common theory is that ergot alkaloids have an oxytocic effect (i.e. contractile response) on the uterus (Saameli, 1978). As previously mentioned, during the late luteal phase oxytocin acts on receptors localized on the endometrium to stimulate synthesis of $PGF_2\alpha$, thus triggering luteolysis (Wathes and Lamming, 1995; McCracken et al., 1999). Only one study has evaluated the effect of ergot alkaloids on $PGF_2\alpha$ secretion. Browning Jr. et al. (1998b) injected ergotamine tartrate to cows during the late luteal phase (day 15 or 16 post-estrus) and observed

that $PGF_2\alpha$ concentrations were elevated just one hour post injection and continued to increase every hour for four hours. This response mirrored that of pulsatile $PGF_2\alpha$ response observed during luteolysis, unfortunately the authors did not evaluate luteal function.

1.5.3 Ergot Alkaloids and Early Embryonic Development

A few studies have evaluated the impact that ergot alkaloids have on oocyte competency and early embryonic development. Jones et al. (2009) predominantly evaluated the fatty acid composition of bovine follicular fluid in heifers grazing either endophyte-free (EF) or endophyte-infected (EI) fescue diets, however they did make few observations on oocyte competency. Abattoir COCs were harvested during the winter months were subjected IVM with a control medium, EF-treated medium with 10% EF plasma, and EI-treated medium with 10% EI plasma supplemented. There were no differences in the percent of oocytes that progressed to MII. Additionally, ovum pick-up was performed on the heifers and the grade I oocytes ($\geq$ 5 layers of compact cumulus cells and homogenous cytoplasm; Hazeleger et al., 1995) were subjected to traditional IVM. Interestingly, there was a difference observed with 66% of the EF grade I oocytes progressing to MII versus 0% of the EI grade I oocytes, thus demonstrating that *in vivo* exposure to endophyte-infected tall fescue can inhibit proper oocyte maturation.

Another study investigated sperm quality (i.e. motility and morphology) and the developmental competence of oocytes fertilized with sperm from bulls grazing endophyte-infected tall fescue. Progressive motility and sperm morphology (i.e. primary and secondary abnormalities) did not differ between bulls grazing EF versus EI. Interestingly, the percent of presumptive zygotes that cleaved was reduced in oocytes fertilized with EI sperm when compared to EF sperm (73.5 vs. 84.0%, respectively). However, this did not impact the percent of cleaved embryos developing to the blastocyst stage between EF and EI treatments (30.1 and 32.4%, respectively; Schuenemann

et al., 2005a). Additionally, a similar study evaluated the same parameters, however rather than grazing they fed a control (CON) diet comprised of cracked corn, corn silage, and soybean meal or an ergotamine tartrate (ET) supplemented diet. Similarly, the percent of presumptive zygotes that cleaved was reduced in oocytes fertilized with ET sperm when compared to CON sperm (51.1 vs. 69.2%, respectively), however, the percent of cleaved embryos developing to the blastocyst stage did not differ between CON and ET treatments (22.2 and 22.0%, respectively; Schuenemann et al., 2005b). Therefore, in both studies the authors speculated that ergot alkaloids could result in ultrastructural damage to sperm that would not be detected using gross morphological diagnostics (i.e. sperm motility and morphology), thus impacting cleavage rates.

The impact of ergot alkaloids on early embryonic development (experiment 1) and uterine receptivity (experiment 2) *in vivo* has additionally been evaluated. Cattle were allotted to receive either the CON or ET diets previously described. In experiment 1, cattle were synchronized and artificially inseminated, and then seven days following estrus, the uterine horn ipsilateral to the CL was flushed for embryo recovery. Embryos were graded for quality (1 being excellent to 4 been degenerated; IETS, 1998) and stage of development. Embryo recovery tended to be more successful in CON cattle versus ET cattle (68.4 vs. 48.7%, respectively). Of the embryos recovered, a greater percent of embryos from CON animals had developed to compacted morula or blastocyst, and there was a greater percent of better quality embryos from CON cattle versus ET cattle (88 vs. 61%, respectively). In experiment 2, seven days following synchronized estrus and AI, two frozen-thawed good quality embryos were transferred to recipients in both treatment groups. Interestingly, pregnancy rates following transfer did not differ (Schuenemann et al., 2005c). The authors concluded that uterine environment is suitable to maintain pregnancy after day 7 of gestation in ET treated animals, however ergot alkaloid exposure appears to detrimentally

affect either the oocyte or the early embryo. It should also be noted that while ADG did not differ in either experiment, rectal temperatures were greater in the ET cattle.

As previously mentioned, elevated body temperature and heat stress-like symptoms are greatly associated with cattle exposed to ergot alkaloids. It is also well established that heat stress can have a negative effect on most aspects of female reproduction including oogenesis, oocyte maturation, and early embryonic development (Hansen, 2009). In fact, results from numerous studies evaluating heat stress and early embryonic development mirror the results observed by Schuenemann et al. (2005a,b,c). For example, Ealy et al. (1993) observed that exposure to heat stress conditions at day 1 post-estrus (2-cell cleaved embryos), reduced the percent of embryos that developed to the blastocyst stage. However, heat stress exposure at days 3, 5, and 7 had no effect on the percent of embryos that were blastocysts. Likewise, Edwards and Hansen (1997) found that heat stress did not impact oocytes during the first 12 hours of maturation *in vitro*, however it did greatly reduce the number of two-cell embryos that developed to the blastocyst stage. Collectively, this may indicate that rather than ergot alkaloid exposure having a direct toxic effect on embryonic development, it may act indirectly via ergot alkaloid-induced heat stress to impact embryonic development and subsequently pregnancy rates.

1.6 The Immune System

The primary function of the immune system is to protect the host from foreign antigens (e.g. microbes, viruses, tumors, and toxins). While the immune system is extremely complex, it can be simplistically categorized as innate (i.e. non-adaptive) immunity and adaptive immunity (Warrington et al., 2011). It is important to remember that innate and adaptive immunity are not mutually exclusive, in fact these systems are complementary in action when protecting the host. There are two predominant methods in which a host can acquire immunity, by either passive or

active immunization. The most common form of passive immunization is maternal passive immunity in which antibody-meditated immunity is transferred to the fetus via the placenta or neonate via colostrum. While active immunization is the production of antibodies against a pathogen post-exposure and the most common example is vaccination (Warrington et al., 2011).

1.6.1 Innate and Adaptive Immunity

The innate immune system is characterized by its immediate response to pathogen exposure by eliciting phagocytosis and an inflammatory response. This response is mediated by a variety of both hematopoietic cells and cytokines (Turvey and Broide, 2010). Hematopoietic cells are derived from white blood cells (i.e. leukocytes) and include macrophages, neutrophils, eosinophils, basophils, and natural killer (NK) cells. Specifically, macrophages and neutrophils play a major role in phagocytosis in which these cells recognize the foreign antigen and engulf it (Alberts et al., 2002a). Additionally, macrophages can synthesize and release numerous cytokines and chemokines in response to inflammation (Cavaillon, 1994; Murray et al., 2005; Stow and Murray, 2013). Cytokines can be classified based on function as either pro- or anti-inflammatory (Cavaillon, 2001). However, numerous cytokines are pleiotropic; therefore, they act on a wide variety of cell types and elicit different effects (Parameswaran and Patial, 2010).

Pro-inflammatory cytokines primarily act to trigger a fever response in order to fight against an infection. These cytokines include, but are not limited to, tumor necrosis factor alpha (TNFα), interferon gamma (IFN-γ), and interleukin (IL)-1 (Cavaillon, 1994; Dinarello, 2000; Cavaillon, 2001). Tumor necrosis factor alpha is one of the most important cytokines mediating inflammatory and innate immune responses. First identified in the 1970s as an endotoxin-induced factor (Carswell et al., 1975), TNFα is a pleiotropic cytokine, but primarily produced by macrophages and signals through two transmembrane receptors, TNF receptor (TNFR) 1 and

TNFR2 (Parameswaran and Patial, 2010; Turner et al., 2014). Activation of TNFR1 results in numerous inflammatory responses, whereas activation of TNFR2 is important for cell proliferation (Parameswaran and Patial, 2010). Collectively, TNFα is a central component in inflammatory cell activation and plays a critical role in the development of chronic inflammatory diseases. Additionally, some pro-inflammatory cytokines can stimulate secondary pro-inflammatory mediators known as chemokines, including chemokine ligand 4 (CCL4) otherwise known as macrophage inflammatory protein (MIP) and chemokine ligand 9 (CXCL9) otherwise known as monokine induced by IFN-γ (MIG; Graves and Jiang, 1995; Griffith et al., 2014).

Anti-inflammatory cytokines regulate pro-inflammatory cytokine response and promote the healing process. These cytokines include, but are not limited to, IL-2, IL-4, IL-10, and IL-13 (Cavaillon, 1994; Opal and DePalo, 2000; Cavaillon, 2001). Interleukin 2 was one of the first cytokines to be characterized and has been extensively investigated due to its effects on the immune system and regulating homeostasis (Gaffen and Liu, 2004). Discovered in 1975 as a growth-promoting activity for bone marrow-derived T lymphocytes (Morgan et al., 1976), IL-2 is a pleiotropic cytokine, but a major function is to promote proliferation of $CD4^+$ and $CD8^+$ T cells, otherwise known as helper and cytotoxic T cells, respectively (Gaffen and Liu, 2004). Some cytokines are redundant in nature, meaning different cytokines can induce the same effect. For example, IL-2 and IL-15 utilize the same receptor and promote proliferation of T cells, however IL-15 is less efficient than IL-2 (Waldmann et al., 2001).

While some cytokines are specific for pro- or anti-inflammatory function, it is not uncommon for cytokines to overlap in roles. Transforming growth factor beta (TGF-β), IL-6, and IFN-α can be classified as either pro- or anti-inflammatory cytokines based on the circumstances (Zhang and An, 2007). Sometimes cytokines and growth factors are used interchangeably.

However, cytokines are defined as polypeptides secreted by leukocytes that act on hematopoietic cells, whereas growth factors are naturally occurring and act on non-hematopoietic cells (O'Shea et al., 2019). Specifically, growth factors stimulate cell differentiation and a classic example would be fibroblast growth factors (FGF1/2) and vascular endothelial growth factors (VEGFs) which stimulate blood vessel formation (i.e. angiogenesis; Cross and Claesson-Welsh, 2001). The classification of some cytokines and growth factors are summarized in Figure 1.1.

Unlike innate immunity which is non-specific, the adaptive immune system has the ability to develop immunological memory which results in enhanced responses to subsequent pathogen exposure. Adaptive immune responses are modulated by two different classes of lymphocytes (i.e. small leukocyte) including the B cells which originate in bone marrow and T cells which originate in the thymus (Alberts et al., 2002b; Bonilla and Oettgen, 2010). The primary function of B cells is to produce antibodies (i.e. immunoglobulins [Ig]) against foreign antigens and this is known as the antibody-mediated immune response (Warrington et al., 2011). Whereas, T cells express antigen-binding receptors on their membrane (i.e. T-cell receptor [TCR]) and are activated by antigen presenting cells (APCs; e.g. dendritic cells) and this is collectively called the cell-mediated immune response (Warrington et al., 2011). There are two classes of T cells: cytotoxic T cells and T-helper (Th) cells. Cytotoxic T cells are directly involved in the destruction of cells infected by the pathogen and are activated by TCR interaction with major histocompatibility complex (MHC) class I proteins. Th cells have no cytotoxic or phagocytic activity, however they help stimulate the responses of other cells and are activated by TCR interaction with MHC class II proteins (Alberts et al., 2002b; Warrington et al., 2011).

1.6.2 Passive and Active Immunization

In mammals such as cattle, intrauterine transfer of maternal antibodies does not occur and the calf must acquire passive immunity via colostrum intake during the neonatal period (Smith and Holm, 1948). Colostrum is defined as the first milk available 24 hours post-parturition and contains all major immunoglobulins (IgG, IgA, and IgM), with IgG being the predominant Ig present (Butler, 1983). Colostrum secretion coincides with a period in time in which the calf's gut permeability allows for the passage of Ig into the circulation, therefore conferring passive immunity (Besser et al., 1988a; Weaver et al., 2000). Typically, high concentrations of IgG in calf serum is an indicator of effective passive transfer and associated with decreased morbidity and mortality due to infectious diseases, such as diarrhea and respiratory disease (Gay, 1984; Donovan et al., 1998). Interestingly, some studies have shown that when cows are vaccinated for bovine rotavirus infection and bovine viral diarrhea (BVD) during late gestation, colostrum intake can also provide passive immunity to these diseases (Besser et al., 1988b; Donovan et al., 2007).

Active immunization is an additional method used to gain immunity and prevent disease, and the most common example is vaccination. Vaccines can provide protection against both viral pathogens including infectious bovine rhiotreachitis (IBR), bovine respiratory syncytial virus (BRSV), bovine viral diarrhea (BVD), and parainfluenza-3 (PI3), and bacterial infections, most commonly *Clostridium*, *Leptospirosis*, and *Vibriosis* (Bagley, 2001; Gunn et al., 2013). There are two categories of vaccines: killed and modified-live. Killed vaccines utilize organisms grown in culture that have been inactivated or "killed" by using chemicals or heat. The advantages of using killed vaccines are that they are less sensitive and more stable in storage, however disadvantages are that they require large amounts of organisms, usually given as a second dose 2-4 weeks later and may not produce as strong, or as long-lasting, immunity (Gunn et al., 2013). Modified-live

vaccines contain very small quantities of a virus or bacteria which have been altered so that they are no longer capable of causing clinical disease, but are capable of infection. Therefore, the host's immune system will replicate the organism and mount an enhanced immune response (Gunn et al., 2013). The major advantage of modified-live vaccines is the wide-range and duration of provided protection, however they must be handled with additional care and some are not labeled for use in pregnant cows due to abortion risks and fetal defects (Bagley, 2001).

For beef calves, the process of weaning is a major stressful event that potentially results in poorer immune response and susceptibility to various diseases (Haley et al., 2005; Lynch et al., 2010). Preconditioning is known as a management practice implemented at the time of weaning and includes vaccination against clostridial and respiratory diseases, with the goal being to enhance immunity and nutritional status of calves while minimizing stress. A few studies have evaluated the impact of preconditioning on subsequent performance. Overall, while there is no difference in cumulative ADG, the ratio of feed to gain (F:G), marbling score, or yield grade in preconditioned compared to non-preconditioned beef calves (Pritchard and Mendez, 1990; Roeber et al., 2001), the percentage morbidity and death loss was reduced during the finishing phase in preconditioned calves (Cole, 1985; Roeber et al., 2001; Lalman et al., 2005). Although the primary value of preconditioning is reducing the risk of subsequent illness in calves, the premium received for preconditioned calves may not always offset the cost associated with preconditioning. Therefore, there have been efforts made to not only improve immunity in beef calves utilizing a preconditioning program, but also subsequent feedlot growth performance and carcass characteristics.

With both vaccination and weaning eliciting an acute-phase immune response, preconditioned beef calves may experience decreased nutrient intake, thus resulting in

compromised growth performance and immune response (Arthington et al., 2013). Therefore, one study evaluated whether the addition of metabolizable protein (MP; 85%, 100%, and 115%) to the diet could improve growth performance and immune response in beef steers. Overall, ADG and F:G was greater for 100% and 115% MP steers compared to 85%. Additionally, serum BVDV-1b titers were greatest in 115% MP steers, indicating a greater immune response. However, preconditioning MP supplementing did not impact finishing growth performance or carcass characteristics (Moriel et al., 2015). A follow-up study evaluated the timing of vaccination relative to weaning (pre- or post-weaning) and weekly post-weaning frequency of energy supplementation (3 times vs. daily) on immune response of beef calves. It was found that the combination of post-weaning vaccination and daily energy supplementation alleviated inflammation and improved overall immune response with the increase of serum BVDV-1b and PI-3 titers (Silva et al., 2018).

1.7 Ergot Alkaloids and the Immune System

While symptoms and various physiological outcomes of fescue toxicosis have been extensity investigated for decades, one area that lacks information is the effect of ergot alkaloids on immunity and response to vaccination. However, it has been suggested that cattle suffering from fescue toxicosis have a suppressed immune system and poor response to vaccination, and thus become susceptible to disease in transit to, and in, the feedlot. Specifically, stocker calves are very susceptible to bovine respiratory disease (BRD) complex. A lack of immunity via failed vaccination potentially due to fescue toxicosis could increase the likelihood that cattle will develop BRD during the stocker or feedlot phase.

1.7.1 Ergot Alkaloids and Innate Immunity

As previously mentioned, the innate immune system is mediated by hematopoietic cells such as macrophages, neutrophils, eosinophils, basophils, and NK cells. Natural killer cells are

populations of lymphocytes that were originally identified by their ability to initiate lysis of tumor growth and were characterized as non-T cells due to the lack of TCR expression (Trinchieri, 1989). Therefore, while some NK cell functions overlap with T cells, NK cells are classified as innate immune responders and produce cytokines such as IFN-γ and TNFα, and chemokines such as MIP (Trinchieri, 1989; Biron, 1997).

One major mechanism by which NK cell activity is regulated is by monoamine neurotransmitters such as serotonin and dopamine (Hellstrand and Hermodsson, 1987; Ricci et al., 1997). Serotonin regulates NK cell function by promoting cell to cell communication with monocytes and IFN-γ secretion via interaction with 5-HT_{1A} (Hellstrand et al., 1993). Additionally, it has been shown that both D_1- and D_2-like receptors are present on lymphocytes (Ricci et al., 1997). Therefore, it was speculated that ergot alkaloids may interfere with serotoninergic and dopaminergic receptors and influence immune responsiveness. It has been demonstrated that NK cell activity is suppressed when PRL concentrations are elevated and NK cell activity is restored by using bromocriptine (i.e. an ergoline derivative and dopamine agonist; Gerli et al., 1986). Conversely, in a PRL-free *in vitro* environment NK cell activity is inhibited when ergolines are added at a 10^{-6}M dose (Fiserova and Pospisil, 1999). Collectively, this indicates that PRL may play a critical role in immune function and that ergot alkaloid exposure could also modulate NK cell function independently of prolactin.

As previously mentioned, TNFα is an important cytokine that mediates inflammatory and innate immune responses. The response of TNFα during inflammation is very similar to the pathophysiology of fescue toxicosis (Thompson and Stuedemann, 1993). An endotoxin called lipopolysaccharide (LPS) is a potent inducer of pro-inflammatory cytokine production and when cattle are administered LPS, plasma TNFα levels increase (Elsasser et al., 1994). Filipov et al.

(1999) was first to report that steers grazing endophyte-infected tall fescue had increased serum TNFα concentrations following an injection of LPS. Additionally, steers grazing endophyte-infected tall fescue had increased serum haptoglobin concentrations 24 hours post LPS injection when compared to steers grazing endophyte-free fescue (Filipov et al., 1999). Haptoglobin is an acute-phase protein predominantly secreted by hepatic cells and is typically elevated in times of great stress such as weaning, feed restriction, and vaccination (Carroll et al., 2009; Arthington et al., 2013; Cappellozza et al., 2014). This suggests that chronic consumption of ergot alkaloids is a stressor that increases pro-inflammatory cytokines which mediate acute phase protein secretion to further contribute to the innate immune response. However, it is important to note that steers on endophyte-infected tall fescue pastures experience a decrease in nutrient intake, and that this can also lead to an immuno-compromised animal. Therefore, because results may be confounded by nutritional status, additional research needs to be completed to elucidate the effect of ergot alkaloids on immune function.

1.7.2 Ergot Alkaloids and Adaptive Immunity

The adaptive immune system is comprised of both B and T cells and provide immunological memory for the host, with B cells being the antibody-mediated immune response and T cells being the cell-mediated immune response (Warrington et al., 2011). It has been previously shown that B cells have α-adrenoceptors that interact with ergot alkaloids and that this will reduce the number of antibodies produced, specifically IgG production (Sterzl et al., 1987; Sula et al., 1991). Notably, Schultze et al., (1999) demonstrated that cattle grazing endophyte-infected tall fescue pastures for two years had reduced serum concentrations of alpha and gamma globulins (i.e. immunoglobulins), indicating long-term immunosuppression.

In contrast to the relationship between PRL and NK cells, a suppression in PRL secretion will in fact diminish immune responses involving macrophages and T cell function (Bernton et al., 1988). As previously mentioned, T cells express TCR and are activated by APCs which are typically dendritic cells, however there is evidence that eosinophils can also function as APCs (Mawhorter et al., 1993). During the initiation of an immune response, IL-4 will activate Th cells via TCR interaction with MHC class II proteins and eosinophils will specifically provide sources of Th_2-inducing cytokines (notably IL-4; auto-regulatory system, Chen et al., 2004; Spencer and Weller, 2010). Interestingly, it has be shown that ergot alkaloid exposure will reduce concentrations of eosinophils, IL-4, and MHC class II proteins (Saker et al., 1998; Oliver et al., 2000; Mayberry, 2018), all of which are key components of the adaptive immune response.

1.7.3 Ergot Alkaloids and Immunization

Only a few studies have evaluated the impact of ergot alkaloid exposure on the effectiveness of immunization. Smejkal-Jagar et al. (1992) administered a single injection of ergot alkaloid dihydroergosine 1 hour post-immunization in rats and observed a suppression in immune reaction. Additionally, Dawe et al. (1997) demonstrated that steers grazing endophyte-infected tall fescue had reduced antibody response when immunized with tetanus toxoid. However, Rice et al. (1997) observed no differences in vaccination responses in steers grazing endophyte-infected versus endophyte-free fescue. Due to the lack of information, more studies need to be completed in order to elucidate the effect of ergot alkaloid exposure has on immune function.

1.8 Mitigating Fescue Toxicosis

Tall fescue is vital to the sustainability of U.S. agriculture and cannot be disregarded, however the negative impact it has on beef cattle performance is an issue that requires a solution. There have been several strategies proposed to mitigate the symptoms of fescue toxicosis including

development of endophyte-free and non-toxic (i.e. novel) endophyte-infected tall fescue varieties, use of intensive pasture management techniques (e.g. inter-seeding pastures with clover and suppression of seed head formation), supplemental feeding, administration of a variety of pharmaceutical remedies (e.g. domperidone), genetic selection, and utilization of the *SLICK* genotype. This review will primarily focus on the endophyte-free and novel endophyte-infected tall fescue varieties, supplemental feeding, and utilization of the *SLICK* genotype. For information regarding the various other methods to mitigate fescue toxicosis refer to Chestnut et al. (1991), Samford-Grigsby et al. (1997), Jones et al. (2003), Aiken et al. (2012), and Smith and Cassady (2015).

1.8.1 Endophyte-Free or Novel Endophyte Varieties

After it was identified that the fungal endophyte was responsible for inducing fescue toxicosis (Bacon et al., 1977), the obvious solution was to develop a fescue variety without the endophyte present (i.e. endophyte-free). The first endophyte-free cultivar, AU-Triumph, was developed and released by the Alabama Agricultural Experiment Station at Auburn University in 1982. This cultivar was developed by combining mass and recurrent selection methods, where mass selection is selecting based off the phenotype from a mixed population and recurrent selection is reselecting generation after generation (Hoveland et al., 1982; Pedersen et al., 1983). Additional methods to eliminate the endophyte is by either short-term heat treatments, long-term storage, or seed applied fungicides (i.e. triazole). For short-term elimination, seeds received a heat treatment of 57°C for 40 minutes or 49°C for 7 days. For long-term elimination, seeds were stored at 21°C for 7 to 11 months (Siegel et al., 1984). While it is not surprising that animal performance is greatly improved on endophyte-free versus endophyte-infected pastures (Buckner et al., 1985), there are numerous agronomic differences. Specifically, endophyte-infected fescue has improved

seedling performance and survival, insect resistance, drought resistance, and greater yield than endophyte-free fescue (Pedersen et al., 1990). Therefore, while pasture renovations are an option and are shown to have long-term economic benefits (Zhuang et al., 2005), it requires extensive management and many producers remain reluctant.

An ideal solution was to identify or modify an endophyte strain that maintained the endophyte associated plant persistence, but did not produce the toxic ergot alkaloids. With collaborating efforts, the patented non-toxic endophyte developed at AgResearch (AR) in New Zealand (Latch et al., 2000) was inserted into seedlings of endophyte-free Jesup and Georgia 5 tall fescue cultivars that were developed at the University of Georgia and several strains were assessed (Bouton et al., 1993b; Bouton et al., 1997; Bouton et al., 2002). Specifically, it was found that the combination of the novel endophyte strain AR542 and the Jesup tall fescue (Jesup MaxQ) resulted in substantially greater animal performance while maintaining similar stand survival and dry matter yield to that of toxic endophyte-infected tall fescue (Bouton et al., 2002). While numerous trials have demonstrated that MaxQ possess both plant persistence and animal performance benefits, renovating existing toxic tall fescue pastures with MaxQ requires extensive effort and with pristine management it would require at least 4 years to cover the establishment costs and produce positive returns on the investment (Gunter and Beck, 2004; Beck et al., 2008).

1.8.2 Supplemental Feeding

With cattle grazing endophyte-infected tall fescue experiencing a decrease in ADG and forage intake (Crawford et al., 1989), it was believed that supplemental feed would increase the rate of gain and improve animal performance. An early study evaluated performance of lambs supplemented with either energy (corn, C) or protein (soybean meal, SBM) while grazing endophyte-infected tall fescue. Lambs without supplementation had a reduced ADG when

compared to either C or SBM supplementation (80, 115, 122 g/d, respectively). Carcass characteristics were also collected and lambs without supplementation had reduced dressing percentages (% = [carcass weight/live weight] x 100) and fat content when compared to either C or SBM supplementation (Daura and Reid, 1991). Similarly, Elizalde et al. 1998 evaluated performance of steers grazing endophyte-infected tall fescue that were supplemented with energy and protein. Supplemented steers had a greater ADG when compared to control steers (0.64 vs. 0.74 kg/d, respectively), however supplementation during the grazing period did not impact subsequent feedlot ADG. Additionally, dressing percentage tended to be reduced for control steers when compared to supplemented steers (58.2 vs. 59.5%, respectively). Together, this may indicate that animals grazing endophyte-infected tall fescue have improved gains when supplemented with energy and/or protein and the supplementation had some carryover effects on carcass composition. However, it is important to note that neither study utilized an endophyte-free control group nor measured the approximate amount of toxins within each pasture.

1.8.3 *SLICK* Genotype

Since tall fescue is predominantly grown in the Southeastern portion of the United States, the elevated temperatures observed in this area will often exacerbate symptoms associated with fescue toxicosis. Typically, *Bos indicus*-influenced cattle, such as Brahman, are common in areas with high environmental temperatures because of their heat tolerance capability (Cartwright, 1980; Turner, 1980). However, there are many problematic features of *Bos indicus*-influenced cattle such as obstinate temperament, late maturing, increased calving interval and poorer carcass quality. Therefore, even if producers are struggling with fescue toxicosis, they are reluctant to incorporate *Bos indicus* genetics to their herd.

Senepol and other Criollo cattle breeds are *Bos taurus*-influenced also have a high heat tolerance due to a slick hair coat. After being introduced into the United States in the 1970s, crossbreeding and selection for slick hair coats to produce heat tolerant animals began. It was observed through these breeding trials that the slick hair coat phenotype was a dominant, inherited trait (Olson et al., 2003). Using a genome scan, Mariasegaram et al. (2008) identified a region (i.e. locus) at approximately 35.0 to 39.4 Mb on bovine chromosome 20 that was suggested to be the locus where the gene responsible for the slick phenotype was located. It was later identified that the prolactin receptor (*PRLR*) located at 39.1 Mb was the gene associated with the slick hair coat phenotype. Specifically, the slick phenotype is a result of a single base deletion in the last exon of the *PRLR* which results in a frameshift mutation and a premature stop codon (Littlejohn et al., 2014). Ultimately, the mutation results in a truncated PRLR protein and shortens the length from 581 amino acids to 461 amino acids, thus removing a substantial portion of the cytoplasmic domain (Porto-Neto et al., 2018). The PRLR is a tyrosine kinase receptor and will dimerize when PRL is bound which subsequently activates the JAK/STAT signaling pathway (Freeman et al., 2000), however it is not known what the impact of the truncation has on the downstream signaling.

A few studies have investigated the thermoregulatory ability of dairy cows with the *SLICK* genotype in heat stress conditions. Slick-haired Holstein cows had reduced vaginal temperatures (39.6 vs. 40.2°C), respiration rates (97 vs. 107 breaths/min.), and greater sweating rates (82 vs. 61 $g.h^{-1}/m^2$) when compared to wild-type Holstein cows, thus indicating that slick-haired cows can more effectively regulate body temperature during acute heat stress conditions (Dikmen et al., 2008). A follow-up study observed similar characteristics and found that slick-haired Holstein cows had improved milk yield during the summer months (May, June, July) when compared to wild-type cows (37.7 vs. 33.7 kg/d; Dikmen et al., 2014). Overall, this demonstrates that the *SLICK*

genotype can alleviate heat stress symptoms and improve overall performance, however no study has directly evaluated the effectiveness of the *SLICK* genotype on combating fescue toxicosis.

1.9 Conclusion

Fescue toxicosis is a multifaceted syndrome that elicits many negative effects on cattle grazing endophyte-infected tall fescue. Specifically, fescue toxicosis severely hinders reproductive performance in cattle and potentially further compromises the immune system during the weaning process, both of which negatively impacts the productivity and profitability of the beef industry. While there have been several strides taken to mitigate the symptoms of fescue toxicosis, these strategies are not cost effective and are only temporary solutions; therefore, beef cattle producers are reluctant to make changes.

The following chapters will not only address the negative outcomes of fescue toxicosis on beef cattle reproduction and immunity, but propose solutions to aid in mitigating various symptoms associated with this syndrome. To do so, our objectives were:

- **Chapter 2:** to evaluate the effect of the slick trait on heifers exposed to ergot alkaloids, specifically describing changes in ovarian function, reproductive hormone secretion, and pregnancy rates.
- **Chapter 3:** to evaluate the effect of supplemental protein on growth and physiological responses of steer calves exposed to ergot alkaloids during the stocker phase.
- **Chapter 4:** to evaluate the effects of supplemental protein and exposure to ergot alkaloids on both innate and adaptive immune responses of vaccinated stocker steers.
- **Chapter 5:** to evaluate the effect of exposure to ergot alkaloids and/or supplemental protein at the time of vaccination on subsequent feedlot performance and carcass traits in steers.

- Finally in **Chapter 6**, we provide an interpretive summary and concluding remarks on fescue toxicosis and beef cattle productivity.

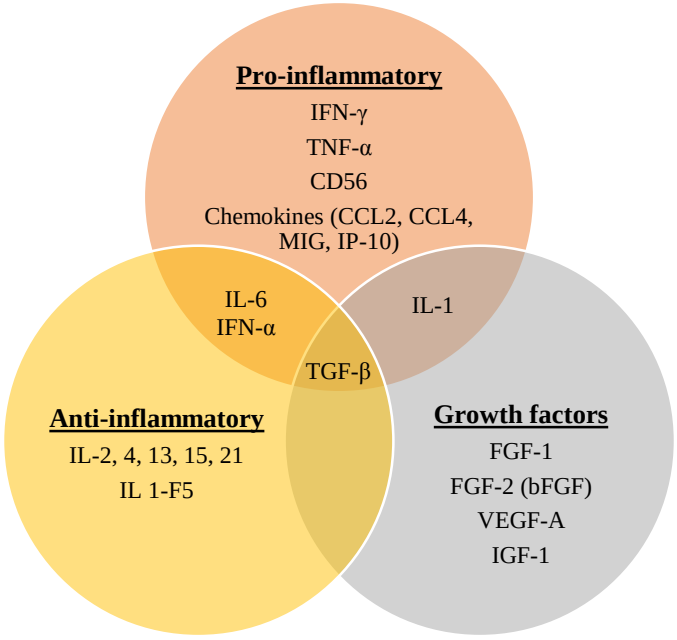

Figure 1.1: Classification of major cytokines and growth factors.

Chapter 2: Impact of slick hair trait on physiological and reproductive performance in beef heifers consuming ergot alkaloids from endophyte-infected tall fescue.

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2.1 Abstract

Fescue toxicosis is a multifaceted syndrome common in cattle grazing endophyte-infected tall fescue. The objective of this study was to evaluate the impact of the slick hair trait on physiological and reproductive parameters in heifers experiencing fescue toxicosis. Angus × Senepol heifers (*n*=31) were blocked by weight (393.5 ± 17.3 kg) and phenotype relative to hair coat at birth, and randomly fed novel endophyte fescue (EN) or endophyte-infected fescue (EI) haylage in a total mixed ration for 91 d. Weekly measurements were collected to monitor heifer growth and response during ergot alkaloids exposure. Following 28 d of treatment, estrus was synchronized and heifers were inseminated. Ovary mapping and AI pregnancy rate were examined via transrectal ultrasonography. Blood samples were taken for genotyping: slick (S) or wildtype (W). Data were analyzed using repeated measures in PROC MIXED of SAS including fescue treatment (EN vs. EI), genotype (S vs. W), and sample collection time as main effects. Body condition scores were decreased for W heifers compared with S heifers (5.48 vs. 5.66, respectively; $P<0.0001$). Surface temperature was greater for EI-W heifers (37.2 °C) compared with other groups (36.4, 36.6, 36.7 °C for EN-S, EN-W, EI-S, respectively; $P<0.05$). Serum PRL concentrations were reduced for EI heifers compared to EN heifers (133.5 vs. 163.1 ng/ml, respectively; $P<0.05$). The average number of 2 to 4 mm follicles were greater in EI-W heifers (13.8 follicles) compared with other groups (12.2, 10.6, and 11.1 for EN-S, EN-W, and EI-S, respectively; $P<0.0001$). However, the average number of preovulatory follicles (≥9 mm) were reduced in EI-W heifers (0.52 follicles)

compared with other heifer groups (0.94, 0.88, and 0.85 ± 0.04 for EN-S, EN-W, and EI-S, respectively; $P<0.05$). Ovulatory follicle size was smaller in EI-W heifers compared with EN-W heifers (9.14 vs. 11.57 mm, respectively; $P=0.05$). Corpus luteum area was reduced in EI-W heifers (235.1 mm^2) compared with other heifer groups (297.2, 272.7, and 276.8 mm^2 for EN-S, EN-W, and EI-S, respectively; $P<0.05$). Concentrations of P4 were greater for EN heifers compared with EI heifers (2.7 vs. 1.8 ng/mL, respectively; $P<0.05$). Pregnancy was not established in EI-W heifers (0%) compared with other heifer groups (37.5%, 57.1%, and 62.5% for EN-S, EN-W, and EI-S, respectively; $P<0.05$). Overall, the slick hair mutation appears to aid in offsetting the physiological symptoms associated with fescue toxicosis and helps to improve reproductive performance.

2.2 Introduction

Tall fescue (*Lolium arundinaceum* [Schreb.] Darbysh.) is a cool-season perennial forage utilized by cow-calf producers in the Southern regions of the United States (Ball et al., 2007). Fescue toxicosis is a result of cattle grazing endophyte (*Epichloë coenophiala*) infected tall fescue, specifically consuming ergot alkaloids produced by the endophytic fungus. This disease contributes to an estimated $2 billion annually in economic loss predominantly due to a reduction in conception rates, which are vital to the success of a cow–calf operation (Kallenbach, 2015).

It has been demonstrated that cattle grazing endophyte-infected tall fescue have impaired reproductive function, including altered ovarian follicle development (McKenzie and Erickson, 1991; Burke and Rorie, 2002) and reduced circulating steroid hormone concentrations (Paterson et al., 1995). A reduction in serum prolactin (PRL) concentrations is a hallmark of fescue toxicosis and is largely regulated by the neurotransmitter, dopamine. The structural similarities between

dopamine and the ergoline ring enables the agonistic effects on the dopamine D2 receptor (Elsasser and Bolt, 1987; Larson et al., 1994), therefore reducing PRL secretion.

In recent years, a genetic trait has been identified in Senepol and other Criollo (*Bos taurus*) cattle breeds that is associated with high heat tolerance and a slick hair coat (Olson, et al., 2003; Mariasegaram et al., 2007; Porto-Neto et al., 2018). A mutation occurs in the prolactin receptor (PRLR) resulting in a slick haired cow (Littlejohn et al., 2014). While the effects of endophyte-infected tall fescue on reproduction have been well documented, the impact of the slick trait has not been investigated. Therefore, the aim of this study is to evaluate the effect of the slick trait on cattle exposed to ergot alkaloids, specifically describing changes in ovarian function, reproductive hormone secretion, and pregnancy rates.

2.3 Materials and Methods

The study was conducted at the Butner Beef Cattle Field Laboratory (BBCFL) in Bahama, NC, and was approved by the Institutional Animal Care and Use Committee at North Carolina State University (13-093-A).

Animals and Treatment

Cattle performance and forage data were collected late May to late August 2015 (see experimental timeline in Figure 2.1). Angus × Senepol heifers (n = 31), with no prior exposure to endophyte-infected fescue, were loaned from CEFS (Cherry Research Farm at the Center for Environmental Farming System in Goldsboro, NC) and delivered to BBCFL in April of 2015. At birth, a phenotype (slick or wildtype) was assigned to each heifer based on technique described by Olson et al. (2003). Heifers were blocked by weight and phenotype then randomly assigned to receive either “toxic” endophyte-infected fescue (EI; 421µg ergovaline/kg of BW) or novel “nontoxic” endophyte fescue (EN; control, 36µg ergovaline/kg of BW) haylage in a total mixed

ration. Towards the end of the experiment, animals were genotyped (Recombinetics, Inc. St. Paul, MN): Slick (S, mutation in PRLR) or Wildtype (W). For analysis, individual treatments were designated as follows: novel endophyte slick (**EN-S**; *n* = 8), novel endophyte wildtype (**EN-W**; *n* = 7), endophyte-infected slick (**EI-S**; *n* = 9), and endophyte-infected wildtype (**EI-W**; *n* = 6). One heifer from EI-S was removed from the study due to the presence of cystic follicles. Animals were placed in a slotted floor barn with individual feeding gates (American Calan, Northwood, New Hampshire) for individual feed intake regulation.

To monitor physiological responses to ergot alkaloids; BW, BCS (scale of 1 to 9; adapted from Whitman, 1975), rectal temperature, surface temperature, heart rate, caudal blood pressure, respiration rate, caudal artery and vein diameter, and hematocrit were measured weekly as previously described in Eisemann et al. (2014). Surface temperature was accessed via thermal imaging camera (Fluke Ti45FT IR Flexcam, Fluke Corporation, Everett, WA). The heifers had an 18x20 cm square clipped behind the left shoulder using a #10 blade. Each week, images were taken within the square and the highest, lowest and average temperature were recorded (SmartView 3.5 Thermal Imager Software; Eisemann et al., 2014). Heart rate and caudal blood pressure were measured three times for each heifer weekly using a 16-24 cm blood pressure cuff (LifeSource A&D Engineering Inc., San Jose, CA). The tail was held steady during these measurements to minimize variation. Caudal artery and vein diameter were measured using Doppler ultrasonography (M-Turbo, SonoSite Inc, Bothell, WA). Hair coat scores (HCS; adapted from Olsen et al., 2003) and hair coat shedding scores (HSS; scale of 1 [slick summer coat] to 5 [full winter coat]; adapted from Gray et al., 2011) were collected by two trained technicians weekly and composited.

For a subset of heifers (*n* = 20; EN-S: *n*=5, EN-W: *n*=5, EI-S: *n*=4, and EI-W: *n*=6), a calibrated iButton temperature data logger was affixed to the bottom side of a progesterone-free controlled internal drug release (CIDR®; Zoetis, Parsippany, NJ) device and inserted into the vagina. The data loggers were set to record internal temperature every 10 minutes for two 7-day intervals during the treatment period (technique adapted from Niu et al., 2014): Period 1 being d 14 to d 21 and Period 2 being d 56 to d 63. Data were blocked into 2 hour intervals for analysis.

Temperature and Temperature Humidity Index (THI)

Ambient temperature and humidity were collected weekly during data collection. Additionally, records were obtained from the National Weather Service Henderson Oxford Airport station, approximately 40 km from BBCFL (Figure 2.2). Temperature-humidity index (THI, Buffington et al., 1977) was calculated using the formula:

$$THI = T_{db} - [0.55-(0.55 \times RH / 100) \times (T_{db} - 58)]$$

where T_{db} represents dry bulb temperature (°F) and RH represents relative humidity.

Diet

Total mixed rations (TMRs) were fed and were based on either Kentucky-31 Tall Fescue (EI, 851 µg/kg total ergot alkaloid and 175 µg/ kg ergovaline) or non-toxic infected tall fescue (MaxQII Texoma, EN, 66 µg/kg total ergot alkaloid and 60 µg/kg ergovaline) haylage. Haylage was harvested by swathing, wilting and chopping, and was preserved in AG bags in the fall of 2014. The TMRs were formulated according to National Research Council (1996) requirements for 0.9 kg/d average daily gain when limited to a dry matter intake of 2% of body weight (71% TDN and 14% CP). DMI was adjusted every 2 wk as the heifers gained weight to maintain the desired rate of gain as described previously (Poole et al., 2018).

The EI TMR was initially 75% haylage, 8% corn, 1.3% soybean meal, 3.1% of a commodity pellet, and 11.9% ground-infected fescue seed (Piedmont Tall Fescue, Southern States Cooperative, Richmond, VA). Midway through the experiment (day 31), a new lot of EI seed was obtained that had a reduced concentration of total ergot alkaloid, so the diet was reformulated to contain 75% EI haylage, 19% ground infected fescue seed, 4% corn, 1% soybean meal, and 0% commodity pellets. The EN TMR contained 63% haylage, 12% corn, 5% soybean meal, and 19% commodity pellets. Both TMRs contained limestone, salt, and a vitamin and trace mineral premix formulated to meet requirements of the heifers. All heifers had ad libitum access to water throughout the experiment.

Infected tall fescue seed was added into the EI TMR to provide a dietary level of 1,000 µg/kg of total ergot alkaloid, while the EN TMR contained 40 µg/kg total ergot alkaloid (Agrinostics, Ltd., Watkinsville, GA). Subsequent analysis of the seed and haylage for ergovaline (MU Veterinary Medical Diagnostic Lab; Rottinghaus, 1993) showed that the EI TMRs contained 421 µg/kg ergovaline, the EN TMR contained 36 µg/kg ergovaline. No ergot alkaloids other than ergovaline were found.

Estrus Synchronization and Reproductive Measurements

Eighteen days after dietary treatment was initiated, all heifers were subjected to the 7-d CO-Synch + CIDR® synchronization protocol. For this protocol, a 100 µg dose of gonadotropin releasing hormone (GnRH, 2 mL Factrel; Zoetis, Parsippany, NJ) was given at the time of CIDR® device (1.38 g of progesterone [P4]; Zoetis) insertion on d 25. At CIDR removal on d 28, a 25 mg dose of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$, 5 mL Lutalyse; Zoetis, Parsippany, NJ) was given followed by a 100 µg dose of GnRH and TAI 72 hours later. Animals were synchronized to display estrus on day 28 of the feeding period (see experimental timeline in Figure 2.1). All heifers were inseminated

to a bull of known fertility and a single inseminator. Daily ovary mapping was performed daily from d 25 to 53 and follicles and corpus luteum (CL) presence were recorded. Follicles were classified based on size: preselected follicles 2-4 mm, transitional follicles 5-8mm, and preovulatory follicles ≥9 mm. Thirty days post-TAI (d 58), pregnancy status was determined by transrectal ultrasonography with a SonoSite M-Turbo ultrasound system equipped with a 10-5 MHz transducer (FUJIFILM SonoSite, Inc., Bothell, WA). Embryo area and crown-to-rump length (CRL) was measured every 5 days from d 58 to 88.

Blood Sampling and Assays

Blood samples were collected from the jugular vein into 10-ml sterile vacutainer serum blood collection tubes without additive (Vacutainer; Becton, Dickinson and Company, Franklin Lakes, NJ). Blood samples were immediately placed on ice and later centrifuged at 1,500 $\times g$ for 20 minutes at 4ºC. The serum was transferred into 5 ml polystyrene vials (BD Falcon, Franklin Lakes, NJ) and stored at -80ºC for analysis.

Serum concentrations of PRL were analyzed on d 0, 14, 28, 42, and 56. Sample concentrations were determined by a commercially available Bovine Prolactin ELISA kit (MyBioSource; San Diego, CA) and previously validated (Poole et al., 2018; Appendix A). A sample control was included in each assay replicate. The inter-assay coefficient of variation based on the duplicate sample controls was 14.45%, and the intra-assay coefficient of variation was 9.93%.

Serum concentrations of estradiol (E2) were analyzed on d 28, 31, 34, 37, 40, 43, 46, and 49 to encompass follicular wave activity throughout the 21 d estrous cycle. Serum E2 was extracted using the procedures of Hendricks et al. (1971). Briefly, 2 ml of ether (Anhydrous Baker Analyzed A.C.S. Reagent, J.T.Baker; Thermo Fisher Scientific Inc., Waltham, MA) was added to 500 µl of

serum in a 16 x 100 mm borosilicate glass tube (VWR, Radnor, PA). Samples were capped and shaken for 10 minutes on an Eberbach Lateral Shaker and frozen at approximately -20°F for 2-3 hours until serum is solidly frozen. Ether supernatant was poured from the frozen serum into a 12 x 75 mm culture tube and placed in a Thermolyne Dry-Bath to evaporate ether from tubes at 45°C used filtered air, followed by adding 50 µl of Steroid Diluent Buffer (MP BioMedicals, Santa Ana, CA) to all tubes. Extracted samples were assayed using the Double Antibody 17β-Estradiol ^{125}I RIA kit (MP BioMedicals, Santa Ana, CA) and were counted for 1 minute using the Micromedic 4/600 Automatic Gamma Counter. The intra- and inter-assay coefficient of variation was 8.63 and 11.39%, respectively.

Serum concentrations of P4 were analyzed on d 28, 33, 38, and 43. Concentrations were determined using Immuchem Coated Tube Progesterone ^{125}I RIA assay (ICN Parmaceuticals, Inc., Costa Mesa, CA) and were counted for 1 minute using the Cobra II Auto Gamma Counter (Packard Instrument Company, Meriden, CT) as previously described by Lyons et al. (2016). The intra- and inter-assay coefficient of variation was 6.12 and 10.42%, respectively.

Serum concentrations of pregnancy-specific protein B (PSPB) were analyzed 28 days post-breeding (d 56) using the commercially available BioPRYN ELISA assay (Biotracking LLC; Moscow ID) as previously described by Mercadante et al. (2016). All samples were run in a single assay and the intra-assay coefficient of variation was 3.88%.

Statistical Analysis

Data were analyzed using the MIXED procedure of SAS 9.3 (SAS Inst. Inc., 1996) with repeated measures and individual animal as the experimental unit. The model for performance data, ovarian measurements, hormone concentrations, pregnancy rates, and embryo measurements included fescue treatment (EN vs. EI), genotype (S vs. W), and sample collection time. Results

were recorded as least squares means ± SEM. Terms with a significance value of $P>0.20$ were removed from the complete model in a stepwise manner to derive the final reduced model for each variable. A statistical significance was reported at a $P\leq0.05$. A tendency was reported at a $P>0.05$ and ≤0.10.

2.4 Results

Animal Performance

All animal performance data is summarized in Table 2.1. There were no differences in average initial BW, final BW, DMI, or ADG. Body condition scores were different by genotype with BCS of W heifers being less than BCS of S heifers (5.48 vs. 5.66 ± 0.03, respectively; $P<0.0001$). As expected, HCS were reduced in S heifers when compared with W heifers (1.94 vs. 3.22 ± 0.09, respectively; $P<0.0001$). Additionally, HSS were decreased in S heifers when compared with W heifers (1.90 vs. 2.84 ± 0.09, respectively; $P<0.0001$). As for fescue treatment effects, EN heifers had decreased HCS when compared with EI heifers (2.37 vs. 2.79 ± 0.11, respectively; $P<0.0001$). Similarly, HSS were reduced for EN heifers when compared with EI heifers (2.14 vs. 2.60 ± 0.08, respectively; $P<0.0001$).

Rectal temperatures were not different among heifer groups ($P>0.05$). Surface temperature was greater in EI-W heifers when compared with other heifer groups ($P<0.05$). Respiratory rates were similar among heifer groups ($P>0.05$). Caudal artery diameter is not different among heifer groups ($P>0.05$). There was an interaction with caudal vein diameter being greater in EI-W heifers when compared with EI-S heifers ($P<0.05$); however, there were no difference when compared with EN-S or EN-W heifers ($P>0.05$). There was an interaction with EI-S heifers having a decreased heart rate when compared with other heifer groups ($P<0.05$). There was a fescue treatment effect with decreased systolic blood pressure in EI heifers compared with EN heifers

(137.5 vs. 145.3 ± 1.1 mmHg, respectively, $P<0.0001$). Similarly, there was an interaction with diastolic blood pressure being reduced in EI-S heifers as opposed to other heifer groups ($P<0.05$). Hematocrit was decreased in EI-W heifers compared with other heifer groups ($P<0.05$).

There was no difference in daily vaginal temperatures in Period 1 (38.7, 38.5, 38.7, and 38.6 ± 0.1 °C for EN-S, EN-W, EI-S, and EI-W, respectively; Figure 2.3a). However, there were differences observed in daily vaginal temperatures with EI-W heifers (39.3 ± 0.2 °C) having significantly greater body temperature compared to other heifer groups in Period 2 (38.6, 38.6, 38.6 ± 0.2 °C for EN-S, EN-W, and EI-S, respectively; $P<0.0001$; Figure 2.3b).

Reproductive Measurements

The average number of preselected follicles (2 to 4 mm) were greater in EI-W heifers compared with other heifer groups ($P<0.0001$). No differences ($P>0.05$) were detected in the average number of selected follicles (5 to 8 mm). The average number of preovulatory follicles (≥9 mm) were reduced in EI-W heifers compared with other heifer groups ($P<0.05$). Additionally, the size of the ovulatory follicle was smaller in EI-W heifers as opposed to EN-W heifers ($P=0.05$). Likewise, CL area was smaller in EI-W heifers compared with other heifer groups ($P<0.05$). Ultimately, there was an interaction observed with heifers in the EI-W group was unable to establish pregnancy as opposed to other heifer groups ($P<0.05$; Table 2.1). No differences were observed in embryo area or CRL between EN-S, EN-W, and EI-S heifer groups (Appendix B; $P<0.05$).

Hormone Profiles

All hormone profile data is summarized in Table 2.2. Overall serum PRL concentrations were reduced for EI heifers compared to EN heifers (133.5 vs. 163.1 ± 10.7 ng/mL, respectively, $P=0.050$). Interestingly, a tendency was observed with S heifers having a reduction in overall

serum PRL concentrations when compared with W heifers (133.8 ± vs. 162.8 ± 10.7 ng/mL, respectively, P=0.06). Moreover, serum PRL concentrations fluctuated throughout the duration of the trial (P<0.001; Figure 2.4). Although there were no fescue treatment differences in overall serum E2 concentrations (5.8 vs. 6.2 ± 0.4 pg/mL for EN and EI, respectively, P=0.45), overall serum E2 concentrations were elevated for S heifers compared with W heifers (7.1 vs. 4.9 ± 0.4 pg/mL, respectively, P<0.001). Overall serum P4 concentrations were elevated for EN heifers compared with EI heifers (2.7 vs. 1.8 ± 0.3 ng/mL, respectively, P<0.05). No differences were observed in the overall P4 concentrations between S and W heifers. Finally, no differences were detected in serum concentrations of PSPB on day 28 of gestation between EN-S, EN-W, and EI-S heifer groups.

2.5 Discussion

The poor performance and reproductive outcomes experienced by EI-W heifers in the present study were remarkably similar to heat-stressed animals. Typically, *Bos indicus*-influenced cattle, such as Brahman, are common in areas with high environmental temperatures (i.e. tropical environment), because of their heat tolerance (Cartwright, 1980; Turner, 1980). However, there are numerous negative features of *Bos indicus*-influenced cattle such as obstinate temperament, late maturing, increased calving interval, and poor carcass quality. Conversely, the Senepol is a *Bos taurus* breed that display similar heat tolerant capabilities yet docile temperament, unlike *Bos indicus*-influenced cattle (Hammond et al., 1996). The heat tolerance capability of Senepol cattle was associated with a slick hair coat which is a dominant, heritable genetic trait (Olson et al., 2003). Specifically, this trait in Senepol cattle is due in part to a frame shift mutation in exon 10 of the prolactin receptor (PRLR) on bovine chromosome 20 (Mariasegaram et al., 2007; Littlejohn et al., 2014). Because of this, it is believed that the Senepol breed can be utilized as a genetic

resource that could reduce problems associated with fescue toxicosis. An experiment conducted by Browning (2004), compared physiological parameters associated with heat tolerance between purebred Hereford and Senepol steers consuming endophyte-infected tall fescue hay. Senepol steers had improved gains, decreased respiration rates, reduced shade use, and reduced skin surface temperatures when compared with Hereford steers, demonstrating the heat tolerance capability of Senepol cattle (Browning, 2004). Similarly in the present study, physiological responses differed between treatment groups. Numerous reports have indicated that cattle consuming ergot alkaloids have a retained and rough hair coat (reviewed by Strickland et al., 2011). While the mechanism of this physiological effect is not well understood, it is known that the retained winter coat exacerbates heat stress during the summer months, and this phenomenon was observed in the EI-W heifers as indicated by the HCS and HSS. Additionally, it has been shown that exposure to ergovaline can result in bioaccumulation within the vasculature, therefore prolonging the effects of ergot alkaloid induced vasoconstriction (Klotz et al., 2009). The combination of a retained winter coat and bioaccumulation of ergovaline most likely contributes to the increased skin surface temperatures experienced by EI-W heifers. While rectal temperature and respiration rate (common responses to heat stress) did not differ between groups, hematocrit values were reduced in EI-W heifers. Hematocrit is the proportion, by volume, of the blood that consists of red blood cells and it is an indicator of dehydration (Nordenson, 2006). Therefore, with EI-W heifers having a decreased hematocrit value, this would indicate that the heifers were overhydrating and attempting to cool down. Moreover, previous studies have shown that cattle consuming endophyte-infected tall fescue have elevated heart rate (Bond et al., 1984), and that additional heat stress exacerbates heart rate (Walls and Jacobsen, 1970). Interestingly, EI-S heifers displayed a decreased heart rate and blood pressure compared with other heifer groups. A decrease in heart rate typically would

result in decreased blood pressure if other variables that affect pressure are unchanged (Melbin and Detweiler, 1993). Therefore, while the EI-S heifers were consuming ergot alkaloids, the heifers displayed a heat tolerance capability which is associated with having a slick hair genotype.

Induction of fescue toxicosis starts to occur ~21 d post-exposure to ergot alkaloids. This is indicated by a reduction in prolactin secretion, urinary excretion of ergot alkaloids, and vasoconstrictive activity (Hill et al., 2000; Klotz et al., 2016). Therefore, while EI heifers had reduced prolactin concentrations 14 d post initiation of the trial, there were no differences among heifer groups in internal data logger temperature (i.e., Period 1). However, as the trial progressed to 56 d, prolactin concentrations normalized between groups, yet EI-W heifers still had a retained, rough hair coat. Thus, only EI-W heifers had significantly increased internal data logger temperature in Period 2. Ultimately as ambient temperatures increased, heifers that possessed the slick hair trait while consuming the endophyte-infected fescue haylage (EI-S) were able to better dissipate heat and have improved overall performance.

In addition to poor performance displayed by cattle suffering from fescue toxicosis, it has been reported that they experience altered follicular dynamics, impaired luteal function, and a reduction in cholesterol and steroid hormone concentrations. Burke et al. (2001) investigated the interaction between heat stress and consumption of endophyte-infected fescue on follicular dynamics. The combination of heifers consuming the endophyte-infected seed in heat stress conditions resulted in a smaller preovulatory follicle diameter and that consumption of endophyte-infected seed alone resulted in a decrease in the number of large follicles (Burke et al., 2001). Similarly in the present study, it was observed that EI-W heifers had a reduction in ovulatory follicle size. Additionally, EI-W heifers had an increase in the number of preselected follicles (2 to 4 mm), however, no change in the number of selected follicles (5 to 8 mm), yet a decrease in

the number of preovulatory follicles (>9 mm). This is indicative of a dysregulation from the selection to preovulatory phase of folliculogenesis. Interestingly, EI-S heifers had similar follicular dynamics to heifers consuming endophyte-free haylage. This would signify that the increase in body temperature due to having a wildtype hair coat, in combination with consumption of endophyte-infected fescue (EI-W), contributes to heat stress and potentially alters the efficiency of ovarian follicular selection and dominance. The concept of heat stress altering folliculogenesis is not novel. A study by Badinga et al. (1993), demonstrated that cows exposed to heat stress conditions did not have a suppression of medium sized (6 to 9 mm) follicles, however did observe smaller preovulatory follicles (Badinga et al., 1993). Additionally, a study completed by Wolfenson et al. (1997) observed that cows subjected to heat stress conditions had decreased 17β-estradiol production by the granulosa cells in the follicular fluid (Wolfenson et al., 1997). Moreover, in the current study, EI-S heifers had elevated serum estradiol concentrations, thus signifying that the ovarian follicles were properly functioning even while consuming endophyte-infected fescue. Together, this indicates that the dysregulation of folliculogenesis and the reduction in steroid hormone concentrations, specifically estradiol, observed in cattle consuming endophyte-infected tall fescue may be solely attributed to the heat stress component of fescue toxicosis.

There have been varying reports regarding the impact of fescue toxicosis on luteal formation and function. Numerous findings report that endophyte-infected fescue reduces CL size and progesterone secretion (Estienne et al., 1990; Mahmood et al., 1994; Burke et al., 2001; Jones et al., 2003), while others observe no differences (Fanning et al., 1992, Seals et al., 2005). This variation in responses could be due to the fact that circulatory ovarian steroid concentrations, specifically progesterone, are not only dependent on the rate of secretion but also on the metabolism in the liver and on the incidence of vasodilation or vasoconstriction. The bovine CL

is a highly vascular tissue that possesses numerous angiogenic factors; however, it has been proposed that vascular endothelial growth factor (VEGF) is responsible for the initial stages of angiogenesis. During the estrous cycle, VEGF concentrations are greatest early in the cycle when luteal vascularization is occurring (Berisha et al., 2000). Interestingly, a hypoxic environment is important for the initiation of VEGF-induced angiogenesis; however, hypoxia will inhibit progesterone secretion mid-cycle (Nishimura and Okuda, 2010). With angiogenic activity occurring in the growing CL, adrenergic receptors will localize in the vasculature and mediate vasoconstriction. Oliver et al. (1998) demonstrated that adrenergic receptors, specifically α2-adrenergic receptors, have a high affinity for endophyte-infected tall fescue produced ergot alkaloid peptides (Oliver et al., 1998). Therefore, the vasoconstrictive activity of ergot alkaloids may potentially be influencing blood flow to the female reproductive tract, specifically the ovary. In the current study, it was observed that EI-W heifers had a reduction in CL area, which would be a direct result of the observed smaller ovulatory follicle size. It has been shown smaller ovulatory follicles will subsequently develop into smaller CL, and secrete less progesterone when compared to larger ovulatory follicles (Vasconcelos et al., 2001). Follicular granulosa cells differentiate into large luteal cells (Smith et al., 1994), and approximately 80% of progesterone secreted is by large luteal cells (Niswender et al., 1985). Therefore, it has been suggested that development of a normal CL depends on the preovulatory follicle having an adequate number of granulosa cells capable of synthesizing sufficient progesterone after luteinization (McNatty et al., 1979). Furthermore, it was observed that EI heifers, regardless of genotype, had reduced serum progesterone concentrations. Together, this signifies the importance of ovarian blood flow, and that the vasoconstrictive activity observed in cattle consuming endophyte-infected tall fescue may be hindering proper ovarian function. Finally, pregnancy was not established in EI-W heifers. The

authors acknowledge the limited dataset and that results may differ will more animals per treatment. However, it is important to note that lower pregnancy rates is a common result of fescue toxicosis and heat stress (reviewed by Porter and Thompson, 1992; Hansen, 2009).

In conclusion, the slick hair mutation observed in Senepol cattle appears to alleviate many of the heat stress symptoms associated with fescue toxicosis and therefore helps to improve reproductive performance. Further research is necessary to elucidate the mechanistic actions responsible for reduced fertility in cattle suffering from fescue toxicosis, specifically evaluating the impact on oocyte competence, fertilization, and early embryonic development.

2.6 Tables

Table 2.1: Physiological parameters for beef heifers consuming either novel endophyte (EN) or endophyte-infected (EI) fescue with slick hair mutation (S) or wildtype (W) from late-May to late-August 2015.

	Treatment[1,2]					*P-value*		
Item	**EN-S**	**EN-W**	**EI-S**	**EI-W**	**SEM**	**Fescue**	**Genotype**	**Interaction**
Initial BW, kg	393.2	388.3	396.9	395.1	17.3	0.762	0.848	0.929
Final BW, kg	422.0	411.2	426.7	417.9	19.0	0.767	0.611	0.960
DMI, kg/d	7.64	7.36	7.49	7.02	0.32	0.447	0.249	0.765
ADG, kg/d	0.46	0.37	0.47	0.36	0.07	0.933	0.156	0.892
BCS[3]	5.70^{a}	5.50^{b}	5.62^{a}	5.47^{b}	0.04	0.146	<.0001*	0.432
HCS[4]	1.64^{a}	3.09^{b}	2.23^{c}	3.35^{b}	0.09	<.0001*	<.0001*	0.073†
HSS[5]	1.59^{a}	2.69^{b}	2.20^{c}	2.99^{b}	0.11	<.0001*	<.0001*	0.149
Rectal temp., °C	39.72	39.72	39.65	39.69	0.04	0.148	0.592	0.534
Surface temp., °C	36.44^{a}	36.64^{a}	36.65^{a}	37.19^{b}	0.10	0.002*	0.004*	0.090†
Heart rate	82.9^{a}	81.4^{a}	75.5^{b}	81.0^{a}	1.5	0.010*	0.192	0.022*
Respiration rate	52.6	52.7	51.3	52.8	1.0	0.541	0.433	0.441
Artery diam., mm	3.22	3.18	3.06	3.17	0.5	0.115	0.526	0.127
Vein diam., mm	4.51^{ab}	4.47^{ab}	4.37^{a}	4.65^{b}	0.8	0.769	0.115	0.041*
Systolic BP[6], mmHg	147.2^{a}	143.5^{a}	136.9^{b}	138.1^{b}	1.5	<.0001*	0.399	0.105
Diastolic BP, mmHg	85.8^{a}	84.7^{a}	75.9^{c}	80.7^{b}	1.4	<.0001*	0.187	0.031*

Table 2.1 (continued).

Hematocrit[7]	36.3[a]	35.2[b]	35.6[b]	33.7[c]	0.3	<.0001*	<.0001*	0.133
Pregnancy rate, %	37.5[ab]	57.1[a]	62.5[a]	0.0[b]	16.8	0.347	0.213	0.021*

[a,b,c] Within row, means without a common superscript significantly differ ($P \leq 0.05$)
[1]Values are reported as least square means for the experiment
[2]EN-S: novel endophyte fescue with slick hair coat; EN-W: novel endophyte fescue with wildtype hair coat; EI-S: endophyte-infected fescue and slick hair coat; EI-W: endophyte-infected fescue with wildtype hair coat
[3]BCS = body condition score (1-9 scale)
[4]HCS = hair coat score (1-5 scale)
[5]HSS = hair shedding score (1-5 scale)
[6]BP = blood pressure
[7]Hematocrit is represented by the percentage of packed red cells in blood
**P-values* <0.05 determined significant
†*P-values* $0.05 > P \leq 0.10$ determined a statistical tendency

Table 2.2: Reproductive parameters in beef heifers consuming either novel endophyte (EN) or endophyte-infected (EI) fescue with slick hair mutation (S) or wildtype (W) from late-May to late-August 2015.

	Treatment[1,2]					*P-value*		
Item	**EN-S**	**EN-W**	**EI-S**	**EI-W**	**SEM**	**Fescue**	**Genotype**	**Interaction**
Follicle Number[3]								
Recruited follicles[4]	12.18[a]	10.60[b]	11.07[ab]	13.77[c]	0.48	0.034*	0.257	<.0001*
Selected follicles[5]	3.09	3.30	3.05	2.96	0.13	0.146	0.663	0.273
Preovulatory follicles[6]	0.94[a]	0.88[a]	0.85[a]	0.52[b]	0.04	<.0001*	<.0001*	0.002*
Ovarian Structure Sizes								
Ovulatory follicle, mm	9.88[ab]	11.57[a]	10.25[ab]	9.14[b]	0.69	0.150	0.674	0.053†
Luteal area, mm^2	297.2[a]	272.7[a]	276.8[a]	235.1[b]	11.7	0.015*	0.005*	0.467
Reproductive Hormones								
Estradiol, pg/ml	6.57[a]	4.97[b]	7.78[a]	4.58[b]	1.82	0.447	0.0001*	0.191
Progesterone, ng/ml	2.38[ab]	3.01[a]	1.97[ab]	1.53[b]	0.46	0.035*	0.837	0.243
Prolactin, ng/ml	140.9[ab]	186.3[a]	124.9[b]	137.2[ab]	31.2	0.050*	0.057†	0.873
PSPB[7], ng/ml	3.36	3.05	3.13	N/A	0.28	0.515	0.430	0.848

[a,b,c] Within row, means without a common superscript significantly differ ($P \leq 0.05$)

[1]Values are reported as least square means for the experiment

[2]EN-S: novel endophyte fescue with slick hair coat; EN-W: novel endophyte fescue with wildtype hair coat; EI-S: endophyte-infected fescue and slick hair coat; EI-W: endophyte-infected fescue with wildtype hair coat

[3]Follicle number is defined as a follicle observed during ovary mapping via transrectal ultrasonography

[4,5,6] Follicles were classified based on size: recruited follicles 2-4 mm, selected follicles 5-8mm, and preovulatory follicles ≥9 mm

[7]PSPB: pregnancy-specific protein B

**P-values* <0.05 determined significant

†*P-values* 0.05>P≤0.10 determined a statistical tendency

2.7 Figures

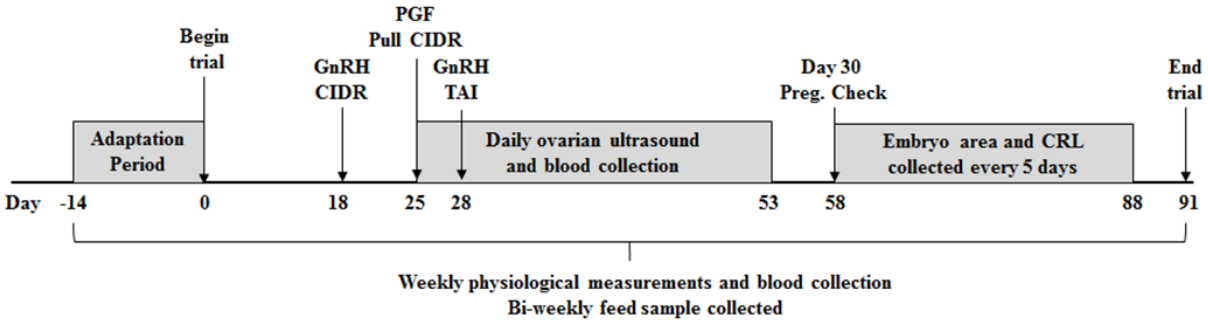


Figure 2.1: Experimental timeline used for beef heifers consuming either novel endophyte (EN) or endophyte-infected (EI) fescue with slick hair mutation (S) or wild-type (W) from late-May to late-August 2015.

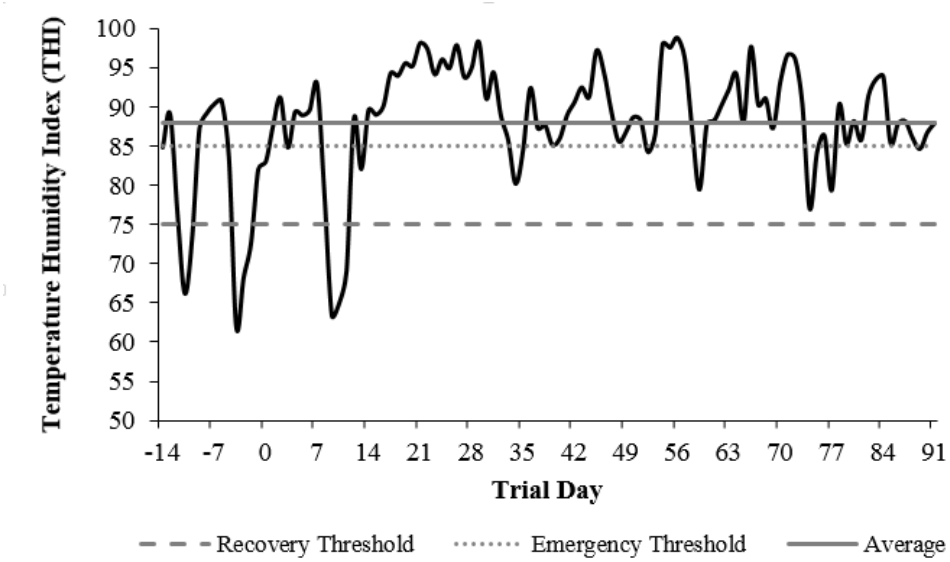


Figure 2.2: Environmental temperature humidity index (THI) retrieved daily at noon from late-May to late August 2015. THI = Tdb – [0.55-(0.55 x RH / 100) x (Tdb – 58)]; Tdb = dry bulb temperature (°F), and RH = relative humidity. Recovery (non-life threatening, 75) and emergency (high-risk, 85) thresholds as described by Hahn (1999). Average represents the overall average THI (88) throughout the duration of the trial.

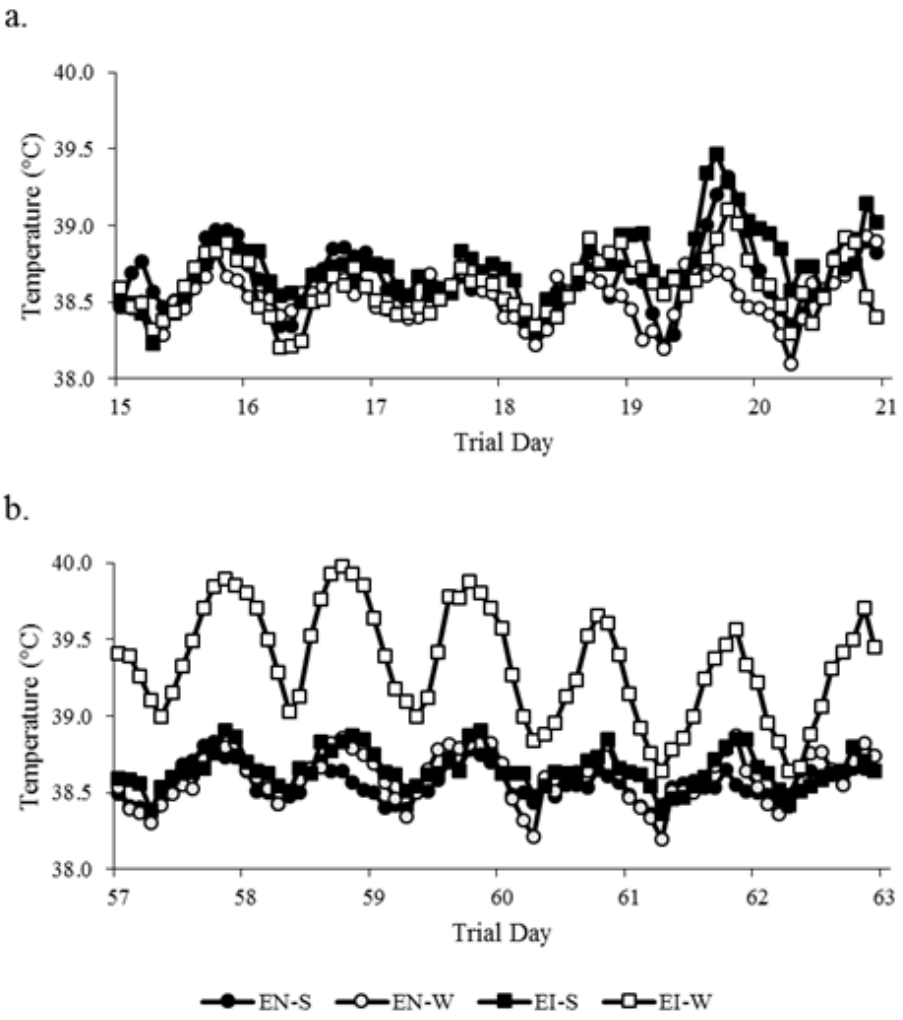


Figure 2.3: For a subset of heifers consuming novel endophyte fescue with the slick hair trait (black circles, EN-S; n = 5) or the wildtype hair coat (open circles, EN-W; n = 5) and endophyte-infected fescue with the slick hair trait (black squares, EI-S; n = 4) or the wildtype hair coat (open squares, EI-W; n = 6), an iButton temperature data logger recorded vaginal temperature every two hours for two 7-day periods: (a) Period 1 being d 14 to d 21 and (b) Period 2 being d 56 to d 63.

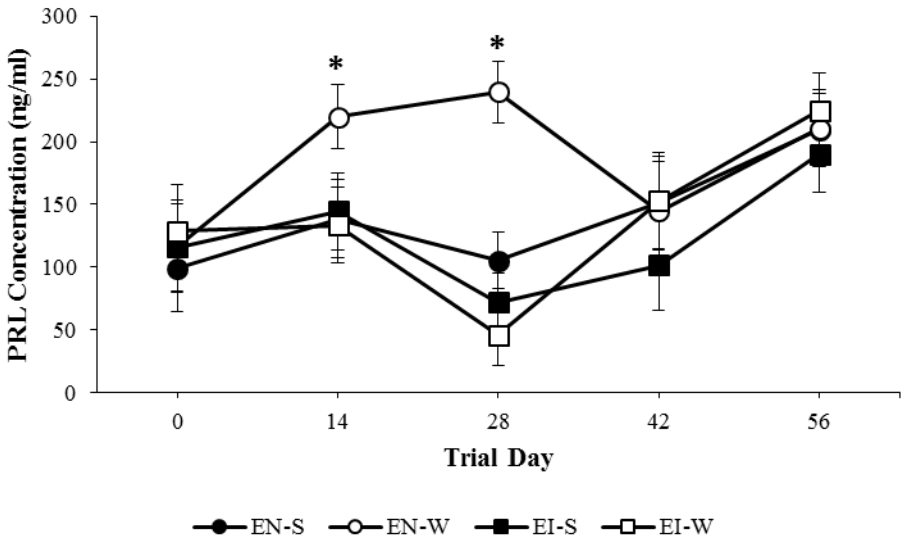


Figure 2.4: Prolactin (PRL) concentrations in heifers consuming novel endophyte fescue with the slick hair trait (black circles, EN-S; n = 8) or the wildtype hair coat (open circles, EN-W; n = 7) and endophyte-infected fescue with the slick hair trait (black squares, EI-S; n = 9) or the wildtype hair coat (open squares, EI-W; n = 6). *P-values <0.05 determined significant.

2.8 Acknowledgements

The authors thank Dean Askew, Greg Shaeffer, and the rest of the Butner crew (Butner Beef Cattle Field Laboratory, Bahama, NC) for their consistent help with animal care and Andrew Meier at CEFS and the North Carolina Department of Agriculture and Consumer Services Cherry Research Farm, in Goldsboro, NC for the use of these heifers. We thank J. Chris Mackey, Sarah M. Long, and Jacob Loyd for all their help with data collection and laboratory analysis. We also thank the North Carolina Cattlemen's Association, North Carolina Agricultural Foundation, Inc., and Hatch Project 02420 for providing the funds for conducting this study.

Chapter 3: Effects of protein supplementation and endophyte-infected tall fescue seed on stocker steers: I. Growth performance and hemodynamic responses.

R. K. Poole, C.M. Womble, M.H. Poore, C. L. Pickworth, and D.H. Poole

3.1 Abstract

Fescue toxicosis is a multifaceted syndrome common in cattle grazing endophyte-infected tall fescue and is detrimental to growth and performance. Recent research has shown that supplementing protein has the potential to enhance growth performance in weaned steers. Therefore, the objective of this study was to evaluate the effect of supplemental crude protein (**CP**) on physiological parameters in stocker steers experiencing fescue toxicosis. Thirty-six weaned Angus steers (6 mo. of age) stratified by weight (196.1 ± 3.6 kg) were assigned to a 2 × 2 factorial arrangement for 56d: endophyte-free seed and 14% CP (**EF-14**; n=9), endophyte-free seed and 18% CP (**EF-18**; n=9), endophyte-infected seed and 14% CP (**EI-14**; n=9), and endophyte-infected seed and 18% CP (**EI-18**; n=9). Steer growth and hemodynamic responses were recovered weekly during ergot alkaloid exposure. On d14 of the trial, iButton® temperature data loggers were subcutaneously inserted in the lateral neck region to record hourly body temperature for 42d. Data were analyzed using PROC MIXED of SAS with repeated measures. No differences were observed in DMI, BW, ADG, F:G, or BCS during the treatment period (P>0.05). Hair coat and hair shedding scores, rectal temperatures, surface temperatures, and respiration rates were greater in EI steers compared to EF steers regardless of protein supplementation (P<0.05). However, subcutaneous body temperature was greater in EI-14 steers (37.94°C) compared to other steer groups (37.60, 37.68, 37.72 ± 0.04°C for EF-14, EF-18, and EI-18, respectively; P<0.05). Heart rate and hematocrit was reduced for EI-18 steers compared to other steer groups (P<0.05). Caudal artery diameter was reduced in EI-18 steers compared to EI-14 steers (2.60 vs. 2.75 ± 0.05 mm,

respectively; $P<0.05$) and caudal vein diameter was reduced in EI-18 steers (3.20 mm) compared to all other steer groups (3.36, 3.39, 3.50 for EF-14, EF-18, and EI-14, respectively; $P<0.05$). However, there was no difference observed in systolic or diastolic blood pressure during the treatment period ($P>0.05$). Based on the data, exposure to ergot alkaloids during the stocker phase had a negative impact on hemodynamic responses and protein supplementation had minimal impact to alleviate symptoms. Therefore, supplementing stocker cattle consuming endophyte-infected tall fescue with additional protein does not seem to be a viable option for beef cattle producers.

3.2 Introduction

Cow-calf operations constitute as the primary beef production system in the Southeast (USDA NASS, 2012). Weaned calves from cow-calf operations are generally placed into a backgrounding program until a sufficient body weight is met for feedlot entry. With forage being the major dietary component for animals in the cow-calf and stocker systems (Allen et al., 2000), stocker production on the same land can improve profitability for Southeastern beef production.

Tall fescue (*Lolium arundinaceum* [Schreb.] Darbysh.) is the predominant forage utilized in the Southeast (Ball et al., 2007), and would be a viable option for stocker grazing. However, fescue toxicosis is a result of cattle grazing endophyte (*Epichloë coenophiala*) infected tall fescue, specifically consuming ergot alkaloids. Vasoconstriction induced by ergot alkaloids interaction with biogenic amide receptors (Klotz et al., 2012; 2013) will reduce blood flow and negatively alter thermoregulation, thus an animal is unable to properly dissipate body heat (Aiken et al., 2007; McClanahan et al., 2008). The exposure to ergot alkaloids during the stocker phase could reduce nutrient intake, leading to compromised performance (Parish et al., 2003). Additionally, the altered

thermoregulation could lead to hyperthermia in elevated ambient temperature, thus exacerbating these negative effects.

Increasing dietary concentration of nutrients, such as protein, can mitigate some of the animal performance lost during periods of reduced feed intake (Elizalde et al., 1998; Moriel et al., 2015). Although calves experience reduced gains while grazing endophyte-infected tall fescue, supplemental protein has been shown to improve growth performance (Elizalde et al., 1998), however there is minimal information pertaining to its impact on thermoregulation. Therefore, the aim of this study is to evaluate the effect of supplemental protein on growth and physiological responses of steer calves exposed to ergot alkaloids during the stocker phase.

3.3 Materials and Methods

The study was conducted at the Butner Beef Cattle Field Laboratory (BBCFL) in Bahama, NC, and was approved by the Institutional Animal Care and Use Committee at North Carolina State University (17-043-A).

Animals and Treatments

Steer performance and feed data were collected early May to early July 2017 (d 0 to56; Figure 3.1). Angus steers (n = 36) grazing on non-toxic fescue pastures, were weaned, at approximately 6 mo. of age, on April 3, 2017 (d -35) and maintained in a dry lot with ad libitum access to non-toxic fescue hay until the start of the trial. Steers were stratified by weight (196.1 ± 3.6 kg) and randomly assigned one of twelve pens (3 steers/pen) within a slotted floor barn. The pens then were randomly assigned to receive either endophyte-infected fescue seed (EI; 185 µg ergovaline/kg of BW) or non-infected "endophyte-free" fescue seed (EF; control, 0 µg ergovaline/kg of BW) and were also randomly assigned to receive either no supplemental protein (100% NRC requirements; 14% crude protein [**CP**]) or soybean meal supplementation (18% CP)

in a total mixed ration. Crude protein levels were selected based on previous research conducted at BBCFL (Moriel et al., 2015). Individual treatments were designated as follows: endophyte-free 14% CP (**EF-14**; n = 9), endophyte-free 18% CP (**EF-18**; n = 9), endophyte-infected 14% CP (**EI-14**; n = 9), and endophyte-infected 18% CP (**EI-18**; n = 9).

To monitor physiological responses to ergot alkaloids; BW, BCS (scale of 1 to 9; adapted from Whitman, 1975), rectal temperature, surface temperature, heart rate, caudal blood pressure, respiration rate, caudal artery and vein diameter, and hematocrit were measured weekly as previously described in Eisemann et al. (2014). Surface temperature was accessed via thermal imaging camera (Fluke Ti45FT IR Flexcam, Fluke Corporation, Everett, WA). The steers had an 18x20 cm square clipped behind the left shoulder using a #10 blade. Each week, images were taken within the square and the highest, lowest and average temperature were recorded (SmartView 3.5 Thermal Imager Software; Eisemann et al., 2014). Heart rate and caudal blood pressure were measured three times for each steer weekly using a 16-24 cm blood pressure cuff (LifeSource A&D Engineering Inc., San Jose, CA). The tail was held steady during these measurements to minimize variation. Caudal artery and vein diameter were measured using Doppler ultrasonography (M-Turbo, SonoSite Inc, Bothell, WA). Hair coat scores (HCS; adapted from Olsen et al., 2003) and hair coat shedding scores (HSS; scale of 1 [slick summer coat] to 5 [full winter coat]; adapted from Gray et al., 2011) were collected by two trained technicians weekly and composited.

On d 14 of the trial, a calibrated iButton temperature data logger was surgically inserted into the lateral neck region of each steer as reported by Lee et al. (2016). Steers were temporary restrained, hair-clipped, and locally anesthetized with 10 ml of 2% lidocaine in the lateral neck regions. A 2 to 3 cm long incision was made in the skin and undermined to make subcutaneous

pocket, thermo-loggers were inserted and then skin was sutured with a medical stapler. The temperature data loggers were recovered by surgery following the procedure above at the end of the feeding period (d 56). The data loggers were set to record the subcutaneous body temperature every hour. Overall retention rate was 86% (EF-14: 8/9, EF-18: 7/9, EI-14: 8/9, EI-18: 8/9). For analysis, subcutaneous body temperature was averaged for the treatment period as well as two 7-day intervals during the treatment period: Period 1 being d 15 to 22 and Period 2 being d 49 to 56.

Temperature and Temperature Humidity Index (THI)

Ambient temperature and humidity were collected weekly during data collection. Additionally, records were obtained from the National Weather Service Henderson Oxford Airport station, approximately 40 km from BBCFL (Figure 3.2). Temperature-humidity index (THI, Buffington et al., 1977) was calculated using the formula:

$$THI = T_{db} - [0.55-(0.55 \times RH / 100) \times (T_{db} - 58)]$$

where T_{db} represents dry bulb temperature (°F) and RH represents relative humidity.

Diet

The 14% CP diets consisted of a corn silage total mixed ration (**TMR**) made up of 61.45% corn silage, 11.75% soybean meal, 13.75% fescue seed (EF or EI), 8.75% ground corn, 3.8% commodity pellets, 0.3% trace mineral salt, and 0.2% limestone on a dry matter (**DM**) basis. The 18% CP diets consisted of 61.45% corn silage, 20.5% soybean meal, 13.75% fescue seed (EF or EI), 3.8% commodity pellets, 0.3% trace mineral salt, and 0.2% limestone on a DM basis. Diets were formulated according to National Research Council (1996) requirements for 1 kg/d ADG at a feed intake of 2.35% of body weight to prevent refusals. Limit feeding to prevent refusals was designed to reduce ergot alkaloid and protein intake variation. Feed offering was adjusted weekly based on average pen BW and silage DM. Cattle were provided ad libitum access to fresh water.

Samples of TMR from each treatment were collected every 28 d, composited, and submitted to be analyzed for nutrient content (Cumberland Valley Analytical Services, Waynesboro, PA; Table 3.1). When refusals were present, they were weighed weekly to calculate actual DMI and visually assessed for presence of fescue seed, in which none was detected.

Tall fescue seed was added into the EI TMR (Piedmont Tall Fescue, Southern States Cooperative, Richmond, VA) to provide a dietary level of 500 µg/kg of total ergot alkaloid, while the EF TMR (Cajun II Tall Fescue, King's Agri Seed Inc., Ronks, PA) contained 0 µg/kg total ergot alkaloid (Agrinostics, Ltd., Watkinsville, GA). Subsequent analysis of the seed for ergovaline (MU Veterinary Medical Diagnostic Lab; Rottinghaus, 1993) showed that the EI TMRs contained 185 µg/kg ergovaline, the EF TMR contained 0 µg/kg ergovaline. Ergovaline was the only ergot alkaloid detected in these samples.

Statistical Analysis

Data were analyzed using the MIXED procedure of SAS 9.3 (SAS Inst. Inc., 1996) with repeated measures and pen as the experimental unit. The model for performance data included fescue treatment (EF vs. EI), protein (14% vs. 18%), sample collection time, and interactions. Results were recorded as least squares means ± SEM. Terms with a significance value of $P>0.20$ were removed from the complete model in a stepwise manner to derive the final reduced model for each variable. A statistical significance was reported at a $P\leq0.05$. A tendency was reported at a $P>0.05$ and ≤0.10.

3.4 Results

Animal Performance

All animal performance data is summarized in Table 3.2. The DMI was uniform across treatments and no differences were observed in BW, ADG, F:G, or BCS. While HCS tended to

differ between fescue treatments (P=0.06), HSS was greater in EI steers compared to EF steers (4.49 vs. 4.36 ± 0.04, respectively; P<0.05). Respiratory rate was increased in EI steers when compared to EF steers (56.5 vs. 52.5 ± 1.6 breaths/min, respectively; P<0.05). Rectal temperatures were elevated in EI steers compared to EF steers (39.7 vs. 39.5 ± 0.06 °C, respectively; P<0.05). Surface temperatures differed between fescue treatments with temperatures being elevated for EI steers compared to EF steers (32.5 vs. 32.2 ± 0.13 °C, respectively; P<0.05). Surface temperatures were also different by protein supplementation with steers fed 14% CP having an increased temperature than steers fed 18% CP (32.6 and 32.1 ± 0.13 °C, respectively; P<0.001). Additionally, there was a treatment interaction with EI-14 steers having greater overall subcutaneous body temperature compared to other steer groups (P<0.05). There was no difference in subcutaneous body temperature by fescue treatment in Period 1 (37.64 vs. 37.74 ± 0.05 °C for EF and EI, respectively; P=0.13; Figure 3.3a). However, there was a difference by fescue treatment in Period 2 with subcutaneous body temperatures being elevated in EI steers compared to EF steers (37.65 vs. 37.39 ± 0.04 °C, respectively; P<0.0001; Figure 3.3b).

Hemodynamic Responses

All hemodynamic measurement data is summarized in Table 3.3. There was a treatment interaction with EI-18 steers having a slower heart rate compared to other steer groups (P<0.05). For caudal artery diameter, there was a treatment interaction with EI-18 steers having a decreased diameter when compared to EI-14 steers (P<0.05), however there was no difference when compared to EF-14 and EF-18 steers (P>0.05). However, caudal vein diameter was decreased in EI-18 steers when compared to all other steer groups (P<0.05). Systolic and diastolic blood pressure did not differ between steer groups (P>0.05). Finally, there was an interaction with

hematocrit being reduced in EI-18 steers when compared to EF-18 and EI-14 steers ($P<0.05$), however there was no difference when compared to EF-14 steers ($P>0.05$).

3.5 Discussion

Retaining calves after weaning for stocker cattle use on pastures has the potential to enhance animal welfare, stocking rates, and the value of calves; thus improving profitability for Southeastern beef production (Allen et al., 1992; Price et al., 2003; Guretzky et al., 2005). With the abundant supply of calves and availability of forage in the Southeast, this would be a viable region for stocker production. Unfortunately, endophyte-infected tall fescue is a prominent forage in this region and has adverse effects on cattle performance (Schmidt and Osborn, 1993; Strickland et al., 2009), thus may negate the value of stocker production. It has been previously shown that fescue seed can reduce ADG even when DMI remains the same in a confinement setting (Poole et al., 2018). However, when feed intake was held at a consistent percent of body weight, fescue seed intake did not alter DMI, ADG, or F:G in the present study. Supplemental protein can improve growth performance of calves post-weaning (Moriel et al., 2015), and improve gains in steers grazing endophyte-infected tall fescue (Elizalde et al., 1998). Contrary to this, the current study showed no protein benefit on DMI, ADG, or F:G in the confinement setting. However, few studies have noted similar observations in beef calves (Smith et al., 1987; Wilson et al., 2014). Therefore, we conclude that by limit feeding, DMI remained the same across treatments and we could accurately evaluate the utilization of nutrients on other performance parameters without the confounding effects of reduced nutrient intake. Furthermore, BCS did not differ between treatment groups. Typically, DMI is reduced in cattle consuming endophyte-infected tall fescue diets (Paterson et al., 1995), thus contributing to loss in body condition. Therefore, because there were no differences observed in DMI, it is not surprising that BCS did not differ.

A common symptom of fescue toxicosis is cattle exhibit a retained and rough hair coat (Strickland et al., 2011). Although the mechanism of this physiological effect is not well understood, it is known that the retained hair coat exacerbates heat stress-like symptoms during the summer months. While there was not drastic differences in hair shedding observed between treatment groups, EI steers did have greater HSS scores when compared to EF steers throughout the trial, with a greater score indicating a retained, rough hair coat. In addition to HSS, EI steers had elevated rectal temperatures and respiration rates compared to EF steers, and both are typical symptoms exhibited in cattle suffering from heat stress and/or fescue toxicosis (Osborn, 1988; Aldrich et al., 1993; Al-Haidary et al., 2001). Specifically, EI-18 steers had a greater respiration rate when compared to both EF steer groups, however EI-14 steers did not differ. In order for cattle to properly dissipate excess body heat during heat stress conditions, the thermoregulatory center within the hypothalamus will trigger a vasodilation response to the peripheral tissues (Curtis, 1983), thus increasing skin surface temperatures. Therefore, EI-14 steers exhibited the highest skin surface temperature of all steer groups and greatest overall subcutaneous body temperature when compared to other steer groups. In combination with lower respiration rates, this would indicate that the EI-14 steers were able to properly dissipate excess body heat. However, skin temperatures were not elevated in EI-18 steers and this group experienced the greatest respiration rate. This would indicate an inability to dissipate body heat via evaporative cooling and that these steers would heavily rely on panting to reduce body temperature. As previously mentioned, EI steers experienced greater HSS and had elevated rectal temperatures, thus displaying heat stress-like symptoms. Typically, cattle under heat stress experience a negative energy balance due to reduced feed intake (Higginbotham et al., 1989), thus would have a reduction in nutrient (i.e. protein) intake. However, in the present study, DMI did not differ between treatment groups, and thus too

much dietary protein could result in excessive conversion of ammonia to urea and/or ammonia toxicity which can have negative effects on numerous biological functions (Visek, 1984). Hence, this could be one explanation for the results observed in the EI-18 steers.

Physiological symptoms of fescue toxicosis arise approximately 21 days post-exposure to ergot alkaloids as indicated by a reduction in prolactin secretion, urinary excretion of ergot alkaloids, and vasoconstriction (Hill et al., 2000; Klotz et al., 2016). Similarly in the present study, there was no difference in subcutaneous body temperature during Period 1 (d 15 to 22 of the trial). In agreement, as the trial progressed the EI steers had significantly increased subcutaneous body temperature during Period 2 (d 49 to 56 of the trial) indicating that the ergot alkaloid from the seed consumed was sufficient to induce fescue toxicosis. Additionally, this phenomenon can be visually observed by the thermal images in Figure 4. Ultimately, this demonstrates that when ambient temperatures begin to increase after fescue toxicosis has been established, signs and repercussions of this syndrome are amplified.

Interestingly, there were no differences in hemodynamic responses (i.e. heart rate, blood pressure, blood vessel diameter, and hematocrit) solely by fescue treatment. However, there were interactions between the fescue and protein treatments on some hemodynamic responses. As the trial progressed, EI-18 steers had significantly lower heart rates when compared to other steer groups. There have been varying reports regarding the impact of fescue toxicosis on heart rate. Numerous findings report that endophyte-infected fescue reduces heart rate (Browning and Leite-Browning, 1997; Aiken et al., 2007; Koontz et al., 2012; Eisemann et al., 2014), while others observe no differences (Rhodes et al., 1991; Poole et al., 2018). However, a reduction in heart rate is typically associated with fescue toxicosis and it has been shown to be independent of ambient temperature. Specifically, Osborn (1988) showed that steers on an EI diet had reduced heart rates

in thermoneutral (21°C; 53 vs. 70 beats per minute [bpm]) and heat stress (32°C; 49 vs. 65 bpm) conditions when compared to steer on EF diets. Therefore, the reduction in heart rate observed by EI-18 can be attributed to fescue toxicosis rather than a rise in ambient temperature throughout the trial. Additionally, EI-18 steers displayed vasoconstriction of the caudal artery and vein compared to EI-14 steers. This is a well-documented symptom of fescue toxicosis (McCollough et al., 1994; Aiken et al., 2007; Poole et al., 2018) and this could indicate a reduction in blood flow to the extremities. While there was no difference observed in blood pressure, this could be explained by the synonymous reduction in both heart rate and blood vessel diameter in EI-18 steers. The final hemodynamic measurement, hematocrit, is the proportion of blood that consists of red blood cells and is an indicator of dehydration (Nordenson, 2006). Hematocrit values were significantly lower for EI-18 steers, specifically when compared to EI-14 steers, and this would indicate that the EI-18 steers were overhydrating in attempt to cool down.

In conclusion, it appears that supplemental protein may not be beneficial to alleviate physiological symptoms of fescue toxicosis when CP requirements are met. In fact, hemodynamic responses worsened in steers fed 18% CP. Taken together, stocker grazing weaned calves on endophyte-infected tall fescue with the addition of supplemental protein may not be viable option for beef cattle producers. However, it is important to note that out in a pasture setting, cattle grazing endophyte-infected tall fescue experience a reduction in nutrient intake and supplementation could ensure that nutrient requirements are met. Therefore, further research is necessary to elucidate if other cost-effective supplemental programs are available to mitigate symptoms of fescue toxicosis and improve stocker grazing on endophyte-infected tall fescue.

3.6 Tables

Table 3.1: Nutritional composition of diets.

	Treatments[1,2,3]			
Item	**EF-14**	**EF-18**	**EI-14**	**EI-18**
DM (%)	43.9	42.3	42.0	42.4
CP (%DM)	13.7	15.3	12.3	15.8
NDF (%DM)	34.0	38.0	35.6	36.8
ADF (%DM)	19.5	23.5	21.4	21.4
TDN (%DM)[4]	71.5	68.8	71.3	69.8
Ash (%DM)	5.00	5.51	4.61	5.45
Ca (%DM)	0.58	0.61	0.53	0.62
P (%DM)	0.34	0.35	0.30	0.34
Mg (%DM)	0.23	0.26	0.23	0.25
Na (%DM)	0.22	0.27	0.23	0.28
K (%DM)	1.01	1.18	1.00	1.18
Cu (ppm)	20	22	19	23
Fe (ppm)	291	307	275	307
Mn (ppm)	55	60	50	55
Zn (ppm)	61	68	58	63

[1]EF-14: endophyte-free 14% crude protein; EF-18: endophyte-free 18% crude protein; EI-14: endophyte-infected 14% crude protein; EI-18: endophyte-infected 18% crude protein
[2]TMR composition for 14% CP (DM basis): 61.45% corn silage, 11.75% soybean meal, 13.75% fescue seed (EF or EI), 8.75% ground corn, 3.8% commodity pellets, 0.3% trace mineral salt, and 0.2% limestone
[3]TMR composition for 18% CP (DM basis): 61.45% corn silage, 20.5% soybean meal, 13.75% fescue seed (EF or EI), 3.8% commodity pellets, 0.3% trace mineral salt, and 0.2% limestone
[4]TDN = 92.5135 – (0.7965 x ADF)

Table 3.2: Physiological parameters for beef steers consuming either endophyte-free (EF) or endophyte-infected (EI) fescue with 14% crude protein (14) or 18% crude protein (18) from early May to early July 2017.

	Treatment[1,2]					*P-value*		
Item	**EF-14**	**EF-18**	**EI-14**	**EI-18**	**SEM**	**Fescue**	**Protein**	**Interaction**
Initial BW, kg	191.0	191.9	195.8	192.5	6.8	0.695	0.863	0.761
Final BW, kg	242.1	245.4	242.7	243.9	7.8	0.957	0.776	0.895
ADG, kg/d	0.91	0.96	0.84	0.92	0.07	0.381	0.355	0.782
DMI, kg/d[3]	5.03	5.10	5.09	5.08	0.05	0.701	0.570	0.541
Feed:Gain[4]	5.68	5.41	6.15	5.60	0.26	0.209	0.125	0.594
BCS[5]	4.88	4.93	4.93	4.93	0.03	0.493	0.379	0.328
HCS[6]	4.54[a]	4.50[ab]	4.61[ac]	4.56[a]	0.04	0.062†	0.286	0.790
HSS[7]	4.36[a]	4.35[a]	4.56[b]	4.42[a]	0.04	0.022*	0.442	0.756
Rectal temp., °C	39.6[a]	39.4[b]	39.7[a]	39.7[a]	0.06	0.005*	0.136	0.392
Surface temp., °C	32.3[a]	32.0[a]	32.8[b]	32.2[a]	0.13	0.011*	0.001*	0.185
SubQ temp., °C[8]	37.60[a]	37.68[ab]	37.94[c]	37.72[b]	0.04	<.0001*	0.068†	0.001*
Respiration rate	52.6[a]	52.3[a]	55.8[ab]	57.2[b]	1.61	0.012*	0.747	0.597

[a,b,c] Within row, means without a common superscript significantly differ ($P \leq 0.05$)

[1]Values are reported as least square means for the experiment

[2]EF-14: endophyte-free 14% crude protein; EF-18: endophyte-free 18% crude protein; EI-14: endophyte-infected 14% crude protein; EI-18: endophyte-infected 18% crude protein

[3]Expressed as average DMI/hd/d

[4]Calculated by dividing DMI/hd/d and ADG

[5]BCS = body condition score (1-9 scale)

[6]HCS = hair coat score (1-5 scale)

[7]HSS = hair shedding score (1-5 scale)

Table 3.2 (continued).

[8]Subcutaneous body temperature averaged from d14 to 56 of the trial
*P-*values* <0.05 determined significant
†P-*values* 0.05>$P \leq 0.10$ determined a statistical tendency

Table 3.3: Hemodynamic responses for beef steers consuming either endophyte-free (EF) or endophyte-infected (EI) fescue with 14% crude protein (14) or 18% crude protein (18) from early May to early July 2017.

	Treatment[1,2]					*P-value*		
Item	**EF-14**	**EF-18**	**EI-14**	**EI-18**	**SEM**	**Fescue**	**Protein**	**Interaction**
Heart rate	93.2[a]	94.8[ab]	98.0[b]	88.0[c]	1.66	0.258	0.003*	0.007*
Caudal artery diam., mm	2.63[b]	2.68[ab]	2.75[a]	2.60[b]	0.05	0.652	0.297	0.028*
Caudal vein diam., mm	3.36[a]	3.39[a]	3.50[a]	3.20[b]	0.06	0.735	0.037*	0.010*
Systolic BP[3], mmHg	143.2	142.0	137.7	142.2	3.3	0.426	0.628	0.386
Diastolic BP, mmHg	63.8	66.3	61.8	59.1	2.9	0.110	0.971	0.371
Hematocrit[4]	33.2[ab]	33.6[a]	34.0[a]	32.7[b]	0.33	0.910	0.128	0.009*

[a,b] Within row, means without a common superscript significantly differ ($P \leq 0.05$)

[1]Values are reported as least square means for the experiment

[2]EF-14: endophyte-free 14% crude protein; EF-18: endophyte-free 18% crude protein; EI-14: endophyte-infected 14% crude protein; EI-18: endophyte-infected 18% crude protein

[3]BP = blood pressure

[4]Hematocrit is represented by the percentage of packed red cells in blood

*_P-values_ <0.05 determined significant

†_P-values_ $0.05 > P \leq 0.10$ determined a statistical tendency

3.7 Figures

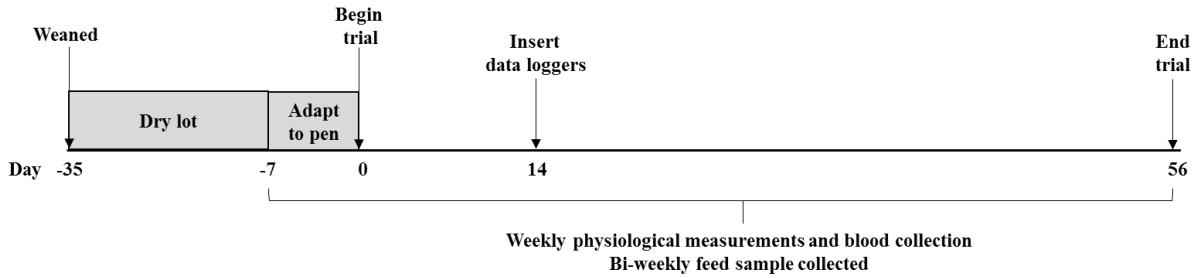


Figure 3.1: Experimental timeline used for steers consuming either endophyte-free (EF) or endophyte-infected (EI) fescue with 14% crude protein (14) or 18% crude protein (18) from early May to early July 2017.

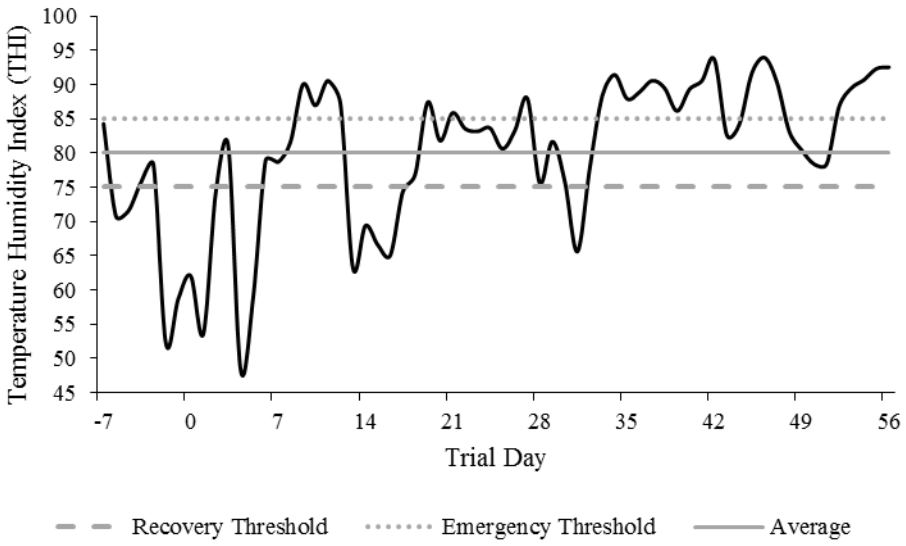
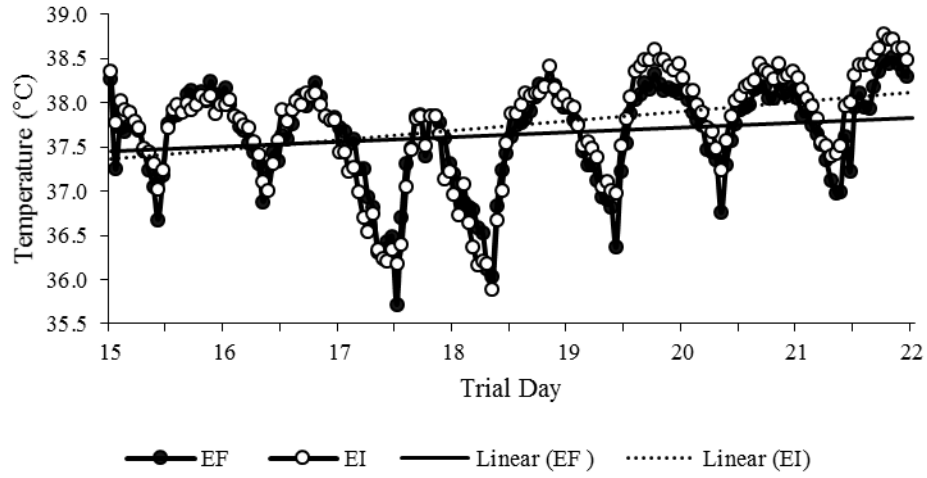


Figure 3.2: Environmental temperature humidity index (THI) retrieved daily at noon from early-May to early July 2017. THI = Tdb – [0.55-(0.55 x RH / 100) x (Tdb – 58)]; Tdb = dry bulb temperature (°F), and RH = relative humidity. Recovery (non-life threatening, 75) and emergency (high-risk, 85) thresholds as described by Hahn (1999). Average represents the overall average THI (80) throughout the duration of the trial.

a.



b.

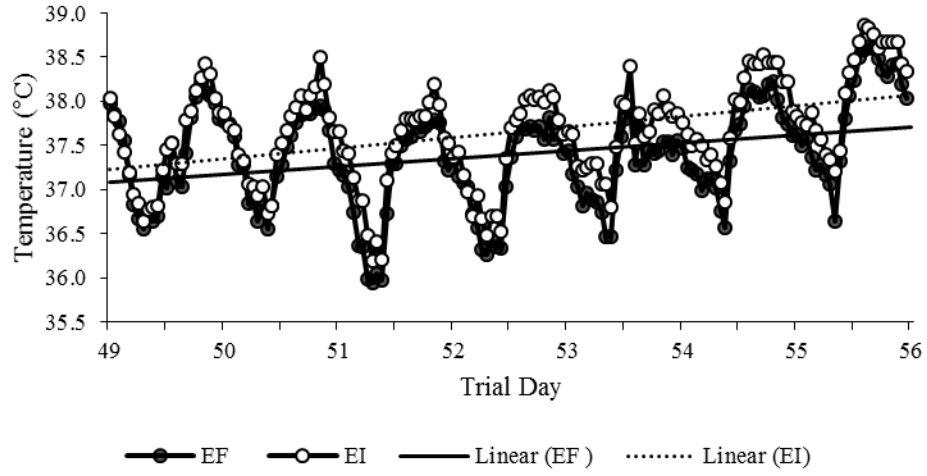


Figure 3.3: Subcutaneous temperatures recorded by iButton temperature data loggers for steers consuming endophyte-free fescue seed (black circles and solid trend line, EF; n = 15) or endophyte-infected fescue seed (open circles and dashed trend line, EI; n = 16) every hour for two 7-day periods: (a) Period 1 being d 15 to 22 and (b) Period 2 being d 49 to 56.

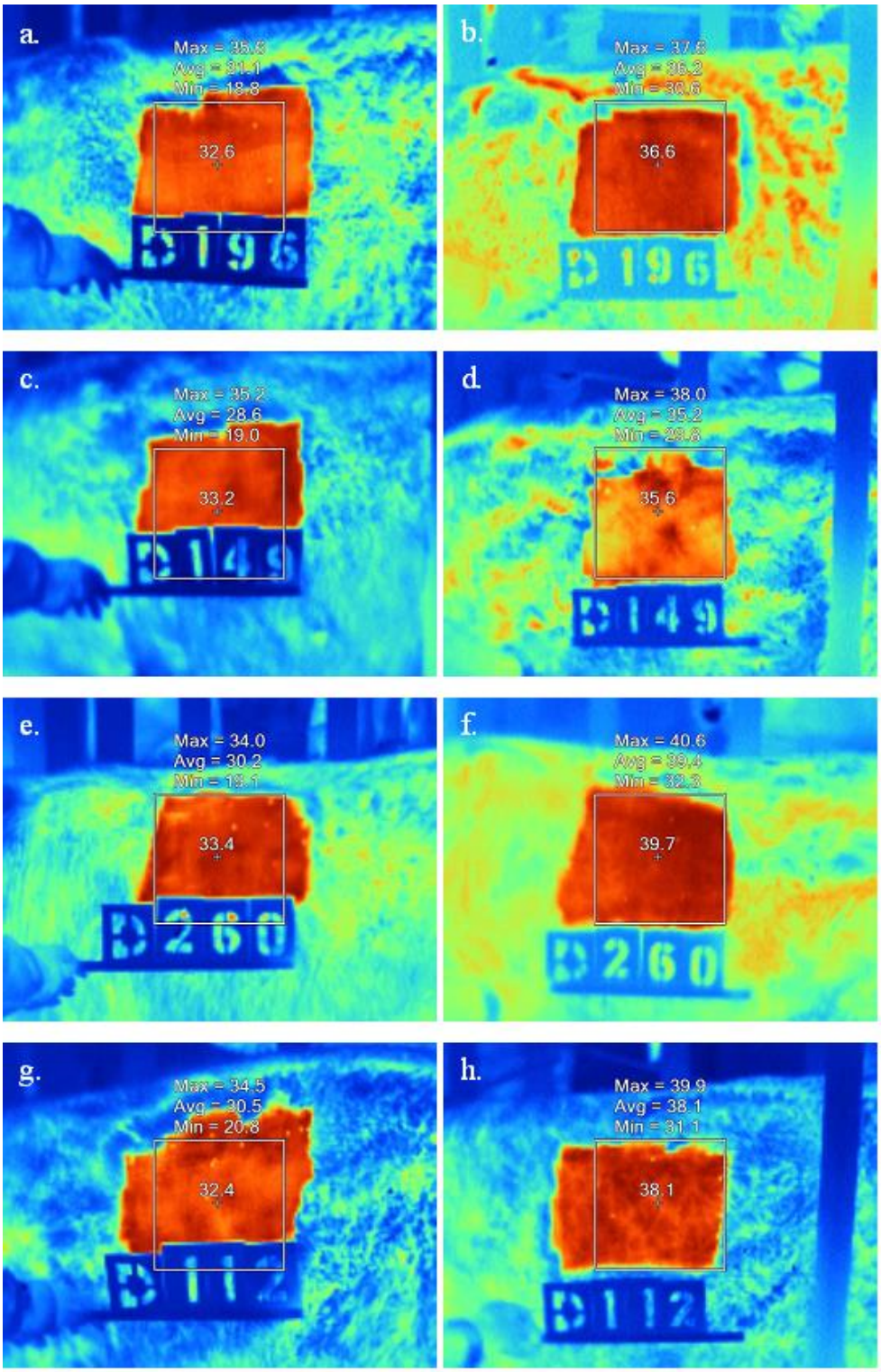


Figure 3.4: Thermal images of surface temperatures for a steer consuming endophyte-free fescue seed with 14% CP (EF-14) on d 0 (a) and 21 (b), endophyte-free fescue seed with 18% CP (EF-18) on d 0 (c) and 21 (d), endophyte-infected fescue seed with 14% CP (EI-14) on d 0 (e) and 21 (f), and endophyte-infected fescue seed with 18% CP (EI-18) on d 0 (g) and 21 (h).

Chapter 4: Effects of protein supplementation and endophyte-infected tall fescue seed on stocker steers: II. Adaptive and innate immune function.

R.K. Poole, A.R. Brown, M.H. Poore, C.L. Pickworth, and D.H. Poole

4.1 Abstract

Fescue toxicosis is a multifaceted syndrome common in cattle grazing endophyte-infected tall fescue that effects growth and performance, however there is little information available pertaining to its effects on immunity. Recently, it has been shown that supplemental protein can improve performance in weaned steers post-vaccination. Thus, the objective of this study was to evaluate the effect of supplemental crude protein (CP) on innate and adaptive immune responses in stocker steers experiencing fescue toxicosis. Thirty-two Angus steers were stratified by weight and treatments were assigned in a 2 × 2 factorial arrangement and included: endophyte-free seed and 14% CP (**EF-14**; n=8), endophyte-free seed and 18% CP (**EF-18**; n=8), endophyte-infected seed and 14% CP (**EI-14**; n=8), and endophyte-infected seed and 18% CP (**EI-18**; n=8). We recently reported no differences in DMI or ADG between treatments, however EI steers exhibited typical signs of fescue toxicosis including elevated rectal temperature and respiration rate. Specifically, EI-14 steers had greater subcutaneous body temperature indicating dissipation of excess body heat. Immune system response was evaluated in steers by vaccinating against infectious bovine rhinotracheitis (IBR) and bovine viral diarrhea virus (BVDV) types 1 and 2 on d 28 of the trial. Steers received a booster two weeks later (d 42). Blood samples were collected at d 0, 42, and 56 to evaluate titers to IBR and BVDV-1b. Additionally, serum cytokine concentrations were evaluated using Quantibody® Bovine Cytokine Arrays on d 0, 28, 31 (72 hr post-vaccine), and 42. Data were analyzed using PROC MIXED of SAS with repeated measures. There were no fescue or protein treatment effects on the seroconversion of IBR and BVDV-1b antibody titers (P>0.05). However, EI steers had greater concentrations of pro-inflammatory cytokines (TNF-α, IFN-α,

IFN-γ, IL-1α), chemokines (CCL2, CCL4, MIG), anti-inflammatory cytokines (IL-2, -13, -15, -21, -36), and various growth factors (FGF-1, IGF-1, VEGF-A) when compared to EF steers (P<0.05). Additionally, EI-14 steers had greater concentrations of VEGF-A and IGF-1 on d 28 of the trial when compared to other steer groups (P<0.05). Based on these data, animals suffering from fescue toxicosis have a hyperactive innate immune response and protein supplementation had minimal impact to mitigate this response. However, it appears that fescue toxicosis had little to no impact on adaptive immunity and response to vaccination.

4.2 Introduction

Weaning is a major stressful event in a calf's life that potentially results in lower immunity and susceptibility to various diseases (Haley et al., 2005; Lynch et al., 2010). Preconditioning is a management practice that is implemented at weaning and includes vaccination against clostridial and respiratory diseases, with the goal being to enhance immunity and performance of calves while minimizing stress. It is believed that providing weaned calves a transition period in a reduced stress environment on forages (i.e. stocker grazing) has the potential to improve performance and profitability before feedlot entry.

Tall fescue (*Lolium arundinaceum* [Schreb.] Darbysh.) is the predominant forage utilized in the Southern regions of the United States (Ball et al., 2007), and would be a viable option for stocker production. However, tall fescue contains an endophyte (*Epichloë coenophiala*) that produce ergot alkaloid toxins, which has adverse effects on beef cattle production and leads to a syndrome known as fescue toxicosis. Exposure to these toxins in stocker cattle critically reduces growth (Parish et al., 2003) and may compromise the immune system by increasing pro-inflammatory cytokine secretion (Filipov et al., 1999), thus counteracting the positive effects of this low-stress transition period.

Increasing dietary concentration of nutrients, such as protein, can mitigate some of the animal performance lost during periods of reduced feed intake, such as while grazing endophyte-infected tall fescue (Daura and Reid, 1991; Elizalde et al., 1998). Additionally, increasing dietary protein can decrease pro-inflammatory cytokine response to endotoxins in beef heifers (Kahl et al., 1997) and during the preconditioning phase improve humoral immune response in steers (Moriel et al., 2015). Therefore, the aim of this study is to evaluate the effects of supplemental protein and exposure to ergot alkaloids on both innate and adaptive immune responses of vaccinated stocker steers.

4.3 Materials and Methods

The study was conducted at the Butner Beef Cattle Field Laboratory (BBCFL) in Bahama, NC, and was approved by the Institutional Animal Care and Use Committee at North Carolina State University (17-043-A).

Animals and Experimental Design

The animals and experimental design used in this study have been previously described (Chapter 3). Briefly, steer performance and feed data were collected early May to early July 2017 (d 0 to 56; Figure 4.1). Due to an infection from data logger incision, a steer from each treatment group was removed from the analysis. Angus steers (n = 32) approximately 6 mo. of age were weaned, stratified by weight, and randomly assigned one of twelve pens. Treatments were fed in a TMR and designated as follows: endophyte-free seed (EF; control, 0 µg ergovaline/kg of BW) 14% CP (**EF-14**; n = 8), endophyte-free seed 18% CP (**EF-18**; n = 8), endophyte-infected seed (EI; 185 µg ergovaline/kg of BW) 14% CP (**EI-14**; n = 8), and endophyte-infected seed 18% CP (**EI-18**; n = 8). Diets were formulated according to National Research Council (1996) requirements for 1 kg/d ADG at a feed intake of 2.35% of body weight to prevent refusals. Limit feeding to

prevent refusals was designed to reduce ergot alkaloid and protein intake variation. Steers did not receive the standard preconditioning (pre-weaning vaccinations), however did receive the pinkeye vaccination with the rest of the herd.

On d 28, all steers were treated with doramectin for internal and external parasites (5 ml subcutaneous [s.c.]; Dectomax injectable, Zoetis Inc., Kalamazoo, MI), and vaccinated against infectious bovine rhinotracheitis (IBR), bovine viral diarrhea viruses (BVDV) types 1 and 2, *Mannheimia haemolytica* (2 ml s.c.; Bovi Shield One Shot, Zoetis Inc.), and *clostridium* (2 ml s.c.; Ultrabac 7, Zoetis Inc.). Two weeks later (d 42), steers received a 2-ml s.c. booster of Bovi Shield Gold 5 (Zoetis Inc.) and Ultrabac 7 (Zoetis Inc.).

Ambient temperature and humidity were collected weekly during data collection. Additionally, records were obtained from the National Weather Service Henderson Oxford Airport station, approximately 40 km from BBCFL. Temperature-humidity index (THI, Buffington et al., 1977) was calculated using the formula:

$$THI = T_{db} - [0.55-(0.55 \times RH / 100) \times (T_{db} - 58)]$$

where T_{db} represents dry bulb temperature (°F) and RH represents relative humidity. Reported in Chapter 3, the overall average THI was 80 throughout the duration of the trial with the recovery (non-life threatening) threshold being 75 and the emergency (high-risk) threshold being 85 as described by Hahn (1999).

Blood Sampling and Assays

Blood samples were collected from the jugular vein into 10-ml sterile vacutainer serum blood collection tubes without additive (Vacutainer; Becton, Dickinson and Company, Franklin Lakes, NJ) and 10-ml sterile vacutainer plasma blood collection tubes with Lithium Heparin

additive (Vacutainer; Becton, Dickinson and Company, Franklin Lakes, NJ). Blood samples were immediately placed on ice and later centrifuged at 1,500 × *g* for 20 minutes at 4ºC. The serum and plasma was transferred into 5 ml polystyrene vials (BD Falcon, Franklin Lakes, NJ) and stored at -80ºC for analysis.

Serum antibody titers against BVDV-1b and IBR viruses for d 0, 42, and 56 were determined by the Oklahoma Animal Disease and Diagnostic Laboratory using a virus neutralization test (Rosenbaum et al., 1970). Serum titers were reported as the $\log_2$ of the greatest dilution of serum that provided complete protection of the cells (lowest and greatest dilution tested = 1:4 and 1:256, respectively). Serum samples with a neutralization value of <4 were considered negative and assigned a value of 0 and samples with a neutralization value of ≥4 were considered positive and assigned a value of 1. These assigned values were then used to calculate seroconversion (% of steers with positive serum neutralization) to BVDB-1b and IBR viruses (Richeson et al., 2008; Artioli et al., 2015; Moriel et al., 2015).

Serum cytokine concentrations were analyzed on d 0, 28 (vaccine), 31 (72 hr post-vaccine), and 42 (booster). Sample concentrations were determined by the Multiplex ELISA Bovine Cytokine Arrays Q1 and Q2 (Quantibody® Cytokine Arrays; RayBiotech, Inc.; Norcross, GA) according to manufactures recommendations. Bovine Cytokine Array Q1 detected the following cytokines and chemokines: interferon alpha (IFN-α), interferon gamma (IFN-γ), interleukin (IL)-13, IL-1α, IL-1-F5, IL-21, and tumor necrosis factor alpha (TNF- α), chemokine ligand 9 (CXCL)-9 (MIG), CXCL-10 (IP-10), and chemokine ligand 4 (CCL4). Bovine Cytokine Array Q2 detected the following cytokines and chemokines: fibroblast growth factor (FGF)-1, basic FGF (bFGF), G Protein-Coupled Receptor Associated Sorting Protein (GPRASP)-1, Insulin-like growth factor (IGF)-1, IL-2, IL-4, IL-15, CCL2, cluster of differentiation (CD) 56, and vascular endothelial

growth factor (VEGF)-A. Serum samples were diluted 1:2 as suggested by the manufacturer. Assays were run as per manufacturer's instructions. Slides were stored at 4°C in a dark box until being sent to the manufacturer for laser scanning and data extraction. Absorbance values were recorded as 'F532 Medium – B532' by the manufacturer and serum concentrations were determined in-house using Q-Analyzer Software for QAB-CYT (RayBiotech, Inc.; Norcross, GA).

Statistical Analysis

Data were analyzed using the MIXED procedure of SAS 9.3 (SAS Inst. Inc., 1996) with repeated measures and animal as the experimental unit. The model for titer response and cytokine concentrations included fescue treatment (EF vs. EI), protein (14% vs. 18%), sample collection time, and interactions. Results were recorded as least squares means ± SEM. Terms with a significance value of $P>0.20$ were removed from the complete model in a stepwise manner to derive the final reduced model for each variable. A statistical significance was reported at a $P\leq0.05$. A tendency was reported at a $P\geq0.05$ and ≤0.10.

4.4 Results

Titer Response

All antibody titer response data is summarized in Table 4.1. A tendency was observed in the seroconversion of BVDV-1b antibody titers with a greater seroconversion in EI-18 steers compared to EF-18 steers ($P=0.09$), no differences were observed when compared to other steer groups. However, there were no differences in the seroconversion of IBR antibody titers. The EI-18 steers had greater serum BVDV-1b titers compared to EF-18 steers on d 0 ($P<0.05$) and compared to all other steer groups on d 42 ($P<0.05$). There were no differences in serum BVDV-1b titers on d 56. Similarly, EI-18 steers had greater serum IBR titers compared to all other steer

groups on d 0 (P<0.05) and compared to EF-18 steers on d 42 (P<0.05). There were no differences in serum IBR titers on d 56.

Cytokine Profiles

All cytokine data is summarized in Table 4.2. The EI steers had greater concentrations of pro-inflammatory cytokines including TNF-α (1.756 vs. 0.689 ± 0.272 ng/ml, respectively), IFN-α (1.027 vs. 0.212 ± 0.262 ng/ml, respectively), IFN-γ (0.347 vs. 0.152 ± 0.061 ng/ml, respectively), and IL-1-F1 (0.319 vs. 0.140 ± 0.060 ng/ml, respectively) when compared to EF steers (P<0.05). However, no differences were observed in the pro-inflammatory cytokine CD56 (P>0.05). The EI steers had greater concentrations of chemokines including CCL2 (140.37 vs. 42.10 ± 23.25 ng/ml, respectively), CCL4 (1.027 vs. 0.212 ± 0.262 ng/ml, respectively), and MIG (1.413 vs. 0.589 ± 0.217 ng/ml, respectively) when compared to EF steers (P<0.05). However, no differences were observed in the chemokine IP-10 (P>0.05). The EI steers had greater concentrations of anti-inflammatory cytokines including IL-2 (14.79 vs. 2.89 ± 3.60 ng/ml, respectively), IL-13 (0.917 vs. 0.380 ± 0.138 ng/ml, respectively), IL-15 (3.56 vs. 1.04 ± 0.59 ng/ml, respectively), IL-21 (1.74 vs. 0.67 ± 0.27 ng/ml, respectively), and IL-1-F5 (0.464 vs. 0.172 ± 0.077 ng/ml, respectively) when compared to EF steers (P<0.05). However, no differences were observed in the anti-inflammatory cytokine IL-4 (P>0.05). The EI steers had greater concentrations in various growth factors including FGF-1 (27.31 vs. 13.53 ± 4.52 ng/ml, respectively), IGF-1 (4.62 vs. 2.81 ± 0.49 ng/ml, respectively), and VEGF-A (90.36 vs. 29.48 ± 12.50 pg/ml, respectively) when compared to EF steers (P<0.05). Moreover, EI-14 steers had greater concentrations of VEGF-A (231.9 vs. 52.5, 26.9, 99.0 ± 30.1 pg/ml for EF-14, EF-18, and EI-18, respectively; Figure 4.2a) and IGF-1 (10.3 vs. 5.8, 5.2, 6.5 ± 1.2 ng/ml for EF-14, EF-18, and EI-18, respectively; Figure 4.2b) on d28 of the trial when compared to other steer groups

(P<0.05), however no differences were observed on d0 or 42 of the trial (P>0.05). There was a tendency for EI steers to have greater concentrations of GPRASP-1 when compared to EF steers (2.87 vs. 2.64 ± 0.09 ng/ml, respectively). Additionally, there was a fescue by protein interaction with EI-18 steers having greater concentrations of FGF-2 when compared to EF-18 and EI-14 steer groups (P<0.05), however not when compared to EF-14 steers (P>0.05).

Finally, there was a decrease in CD56 concentrations 72 hr post-vaccination in EF-14, EF-18, and EI-14 steers (P<0.05), however no differences were observed in EI-18 steers (P>0.05; Figure 4.3a). There was also a decrease in IGF-1 concentrations 72 hr post-vaccination in EI-14 steers (P<0.05), however no differences were observed in other steer groups (P>0.05; Figure 4.3b). There were no other cytokines that differed 72 hr post-vaccination (P>0.05).

4.5 Discussion

The process of weaning and vaccination has been documented to elicit an acute-phase immune response, therefore preconditioned beef calves may experience decreased nutrient intake, thus resulting in compromised growth performance (Carroll et al., 2009; Arthington et al., 2013). In addition to these stressors, cattle grazing endophyte-infected tall fescue also experience reduced gains and poor growth performance (Schmidt and Osborn, 1993; Strickland et al., 2009), which has been attributed to a decrease in nutrient or dry matter intake (Paterson et al., 1995; Matthews et al., 2005). Therefore, the combination of weaning stress and exposure to endophyte-infected tall fescue, specifically the ergot alkaloid toxins, leads to a reduction in nutrient intake and could potentially compromise immune function in stocker calves. However, it has been shown that supplemental protein can improve both growth performance and immune response in steers during the preconditioning period (Moriel et al., 2015), and improve gains in steers grazing endophyte-infected tall fescue (Elizalde et al., 1998). To reduce the confounding effects feed intake has on

performance and immune function, all treatment groups in the present study were given feeding regiments that controlled daily intake and ensured that nutrient requirements were met. Therefore, there were no differences observed in DMI or ADG between steer groups (Chapter 3), thus all differences observed in immune response can solely be attributed to consumption of ergot alkaloids and/or protein supplementation.

Detection of neutralizing antibody titers provides an assessment of an animal's defense mechanism for viral infection and vaccination efficiency (i.e. adaptive immunity, Bolin and Ridpath, 1990; Fulton et al., 1997). Response to vaccination varies among animals and is dependent on numerous factors, such as environment, genetics, timing of vaccination, and maternal antibody concentrations (Kirkpatrick et al., 2008; Glass et al., 2012; Downey et al., 2013). However, minimal research has been completed to investigate the impact of nutrition on vaccine response. In the present study, all steer groups had the presence of serum antibody titers for both BVDV-1b and IBR on d 0 (pre-vaccine) with EI-18 steers having the greatest amount present, thus indicating that the steers have previously been exposed to both viruses. However, it has been shown that a calf can mount an overall antibody response to vaccination if circulating antibodies are below 3.12 (log_2; Downey et al., 2013). This was observed in the present study with a positive seroconversion of both BVDV-1b and IBR for all steer groups, with EI-18 steers having the greatest seroconversion most likely due to having the greatest amount of titers present on d 0. Therefore, chronic exposure to ergot alkaloids does not appear to negatively impact adaptive immune responses to vaccination and there appears to be no benefit to supplemental protein. In contrasts to this, Moriel et al. (2015) suggested that supplemental protein during vaccination does increase antibody production against BVDB-1b which may enhance immune protection. However, unlike the experimental design in the current study, steers with supplemental protein had

significantly greater DMI and ADG (Moriel et al., 2015), thus suggesting that the increase in antibody production was a result of increased nutrient intake and enhanced growth performance.

Filipov et al. (1999) was the first to report that steers grazing endophyte-infected tall fescue had increased serum TNF-α concentrations following an injection of lipopolysaccharide (LPS, potent inducer of pro-inflammatory cytokines; Elsasser et al., 1994). However, it was also reported that these steers had reduced BW and ADG compared to their endophyte-free counterparts, thus suggesting that a reduction in nutrient intake, which can itself result in an immuno-compromised animal (Cappellozza et al., 2014). In the current study, exposure to ergot alkaloids without the confounding effect of nutrient restriction, resulted in elevated levels of the pro-inflammatory cytokines TNF-α, IFN-α, IFN-γ, and IL-1-F1. These cytokines primarily act to mediate innate immunity and trigger a fever response in order to fight against an infection. Additionally, some pro-inflammatory cytokines (most commonly TNF-α) can stimulate secondary pro-inflammatory mediators known as chemokines which are responsible to stimulate the recruitment of leukocytes (white blood cells) to the site of infection (Graves and Jiang, 1995). In the present study, steers consuming ergot alkaloids had increased levels of the chemokines CCL2, CCL4, and MIG. Many symptoms of fescue toxicosis, such as elevated core body temperature and compromised thermoregulation (hyperthermia), are very similar to a fever response or inflammation, and would likely involve pro-inflammatory cytokines as suggested by our data. Anti-inflammatory cytokines regulate pro-inflammatory cytokine response and promote the healing process. Because pro-inflammatory cytokines were elevated, it is not surprising that anti-inflammatory cytokines including IL-2, -13, -15, -21, and -1-F5 were also elevated in steers consuming ergot alkaloids. Together, it appears that chronic exposure to ergot alkaloids results in a hyperactive innate immune

response similar to inflammation and may lead to an immuno-compromised animal which would be at high risk of susceptibility to bovine respiratory disease (BRD) complex.

Growth factors stimulate cell differentiation and proliferation. In the present study, steers consuming ergot alkaloids had greater concentrations of growth factors including FGF-1, IGF-1, and VEGF-A. These growth factors are very important for angiogenesis and vessel vasodilation (Cross and Claesson-Welsh, 2001). Potentially, the increase in these angiogenic factors following exposure to ergot alkaloids may be to counteract the negative effects of fescue toxicosis has on vascularity. Moreover, the current study demonstrates that EI-14 steers have an increase in both VEGF-A and IGF-1 from d 0 to 28 of trial. This would be in agreement with the skin surface temperature and subcutaneous body temperature results in Chapter 3. The EI-14 steers exhibited the highest temperatures indicating peripheral vasodilation, likely due to an increase in VEGF-A and IGF-1, to dissipate excess core body heat.

In addition to these growth factors, steers consuming ergot alkaloids had greater concentrations of GPRASP-1. This is a protein that has been shown to interact with numerous G-protein coupled receptors including the dopamine-D2 receptor. This interaction down-regulates D2 receptors and preventing the receptor to resensitize to agonist treatment (Bartlett et al., 2005). When dopamine or an agonist is bound to the receptor, prolactin secretion is suppressed (Enjalbert and Bockaert, 1983; Lledo et al., 1992). Ergot alkaloids have structural similarities to numerous biogenic amines such as dopamine (Berde, 1980), therefore would have an agonistic effect, resulting in a decrease in prolactin secretion (Elsasser and Bolt, 1987; Larson et al., 1995). Prolactin concentrations have been shown to be reduced in animals exposed to ergot alkaloids and this is often used as an indicator of fescue toxicosis (Strickland et al., 2011). Additionally, a suppression in prolactin secretion will in fact diminish immune responses by immune cells, such

as macrophages and T cell function (Bernton et al., 1988). Therefore, this increase in GPRASP-1 concentrations in EI steers is could be a biological defense mechanism to down-regulate D2 receptors to prevent a decrease in prolactin secretion and immune function.

Typically, an innate immune response to vaccination subsides approximately 3 days (72 hours) post-exposure and signals activation of the adaptive immune system (Chaplin, 2010), which aids in the develop of immunological memory for enhanced responses to subsequent pathogen exposure. To combat viruses, the immune system has two primary strategies: generation of cytotoxic T cells and NK cells. Cytotoxic T cells are directly involved in the destruction of cells infected by a pathogen and are activated by T-cell receptor (TCR) interaction with major histocompatibility complex (MHC) class I proteins (Warrington et al., 2011). However, some complex viruses can down-regulate expression of MHC-class I proteins, therefore NK cells evolved to further protect the host. Natural killer cells were originally identified by their ability to lysis tumor growth, but were characterized as non-T cells due to the lack of TCR expression (Trinchieri, 1989). However, NK cells do express phenotypic markers on the cell surface called neural cell adhesion molecule (NCAM), also known as CD56. While the reason NK cells express CD56 remains unanswered, it is known that CD56 expression coincides with NK cell activation (Van Acker et al., 2017). In the present study EF-14, EF-18, and EI-14 steer groups had decreased CD56 concentrations 72 hours post-vaccination, however concentrations remained unchanged in EI-18 steers. This could indicate that the innate immune system was still activated in EI-18 steers in response to the vaccine. Additionally, EI-14 steers had decreased IGF-1 concentrations 72 hours post-vaccination. Primarily secreted by the liver in response to growth hormone, IGF-1 activates cell proliferation and differentiation, tissue growth and development, and has anti-inflammatory properties (Lara-Diaz et al., 2017). However, it has been shown that pro-inflammatory cytokines

can induce a state of IGF-1 resistance, which would inhibit the effects of IGF-1 and facilitate energy and protein mobilization from body stores (O'Connor et al., 2008). The elevated levels of the pro-inflammatory cytokines observed in EI steers could have induced a state of IGF-1 resistance, however this phenomenon was not observed in EI-18 steers potentially due to the provided supplemental protein.

In summary, exposure to ergot alkaloids does not appear to negatively impact adaptive immune responses, but does appear to result in a hyperactive innate immune response and supplemental protein has minimal impact to negate this response. However, when CP requirements are met and steers exposed to ergot alkaloids are provided additional protein at the time of vaccination, this may aid in alleviating muscle protein mobilization and prevent any stress-induced weight loss.

4.6 Tables

Table 4.1: Seroconversion and serum concentrations of antibody titers against bovine viral diarrhea virus type 1b (BVDV-1b) and infectious bovine rhinotracheitis (IBR) in steers consuming either endophyte-free (EF) or endophyte-infected (EI) fescue with 14% crude protein (14) or 18% crude protein (18) during a 56 d treatment period[1].

	Treatment[2,3]					*P-value*		
Item	**EF-14**	**EF-18**	**EI-14**	**EI-18**	**SEM**	**Fescue**	**Protein**	**Interaction**
BVDV-1b								
Seroconversion, %	59.3[ab]	48.2[a]	51.9[ab]	66.7[b]	7.5	0.462	0.806	0.088†
Serum titers, log_2								
d 0	2.40[ab]	1.47[a]	1.69[ab]	2.96[b]	0.57	0.497	0.770	0.061†
d 42	0.29[a]	0.00[a]	0.00[a]	2.62[b]	0.55	0.041*	0.041*	0.012*
d 56	2.96	3.51	3.13	3.73	0.68	0.771	0.403	0.974
IBR								
Seroconversion, %	51.9	44.4	51.9	66.7	9.3	0.233	0.690	0.233
Serum titers, log_2								
d 0	1.67[a]	1.40[a]	1.67[a]	3.29[b]	0.57	0.108	0.244	0.108
d 42	1.00[ab]	0.22[a]	0.44[ab]	1.78[b]	0.52	0.342	0.596	0.050*
d 56	1.73	1.56	1.78	3.07	0.57	0.185	0.340	0.210

[a,b] Within row, means without a common superscript significantly differ ($P \leq 0.05$)

[1]On d 28, all steers were vaccinated against infectious bovine rhinotracheitis (IBR), bovine viral diarrhea viruses (BVDV) types 1 and 2, *Mannheimia haemolytica* (2 ml s.c.; Bovi Shield One Shot, Zoetis Inc.), and *clostridium* (2 ml s.c.; Ultrabac 7, Zoetis Inc.). On d 42, steers received a 2-ml s.c. booster of Bovi Shield Gold 5 (Zoetis Inc.) and Ultrabac 7 (Zoetis Inc.).

[2]Values are reported as least square means for the experiment

Table 4.1 (continued).

[3]EF-14: endophyte-free 14% crude protein; EF-18: endophyte-free 18% crude protein; EI-14: endophyte-infected 14% crude protein; EI-18: endophyte-infected 18% crude protein

*P-*values* <0.05 determined significant

†P-*values* $0.05 > P \leq 0.10$ determined a statistical tendency

Table 4.2: Serum concentrations of cytokines (pro-inflammatory, anti-inflammatory, and chemokines) and growth factors (reported in ng/ml unless otherwise stated) for beef steers consuming either endophyte-free (EF) or endophyte-infected (EI) fescue with 14% crude protein (14) or 18% crude protein (18) from early May to early July 2017.

	Treatment[1,2]					*P-value*		
Item	**EF-14**	**EF-18**	**EI-14**	**EI-18**	**SEM**	**Fescue**	**Protein**	**Interaction**
Pro-inflammatory								
TNF-α	0.793[a]	0.583[a]	1.802[b]	1.710[b]	0.379	0.007*	0.696	0.879
IFN-α	0.155[a]	0.269[a]	0.872[b]	1.182[b]	0.364	0.030*	0.568	0.791
IFN-γ	0.183[ab]	0.121[a]	0.298[ab]	0.396[b]	0.084	0.025*	0.834	0.351
IL-1-F1[3]	0.182[ab]	0.097[a]	0.360[b]	0.278[ab]	0.083	0.037*	0.326	0.983
CD56	25.21	28.36	23.33	22.04	2.63	0.130	0.728	0.411
Chemokines								
CCL2	48.14[a]	36.07[a]	186.78[b]	93.96[a]	32.31	0.004*	0.115	0.223
CCL4	0.106[a]	0.146[ab]	0.418[ab]	0.477[b]	0.151	0.040*	0.750	0.952
MIG	0.553[a]	0.626[a]	1.725[b]	1.100[ab]	0.302	0.009*	0.372	0.259
IP-10	0.666	0.470	0.691	0.690	0.095	0.206	0.309	0.315
Anti-inflammatory								
IL-2	2.80[a]	3.00[a]	8.80[a]	20.77[b]	5.00	0.022*	0.236	0.251
IL-4	4.02	0.32	0.90	0.33	2.03	0.454	0.306	0.452
IL-13	0.448[a]	0.313[a]	0.770[ab]	1.064[b]	0.192	0.008*	0.687	0.277
IL-15	0.844[a]	1.229[a]	4.792[b]	2.334[a]	0.819	0.003*	0.217	0.092†
IL-21	0.782[a]	0.558[a]	2.022[b]	1.452[ab]	0.369	0.006*	0.293	0.646

Table 4.2 (continued).

IL-1-F5[4]	0.212[ab]	0.132[a]	0.460[b]	0.469[b]	0.108	0.009*	0.746	0.687
Growth factors								
FGF-1[5]	11.25[a]	15.82[ab]	31.70[b]	22.92[ab]	6.29	0.034*	0.743	0.300
FGF-2[6,7]	6.41[ab]	3.10[a]	3.38[a]	8.44[b]	1.56	0.480	0.590	0.012*
IGF-1	2.82[a]	2.79[a]	4.72[b]	4.53[ab]	0.69	0.011*	0.878	0.915
VEGF-A[7]	29.27[a]	29.69[a]	114.84[c]	65.88[b]	17.37	0.001*	0.174	0.166
GPRASP-1	2.67[ab]	2.61[a]	2.96[b]	2.77[ab]	0.15	0.093†	0.358	0.629

[a,b,c] Within row, means without a common superscript significantly differ ($P \leq 0.05$)
[1]Values are reported as least square means for the experiment
[2]EF-14: endophyte-free 14% crude protein; EF-18: endophyte-free 18% crude protein; EI-14: endophyte-infected 14% crude protein; EI-18: endophyte-infected 18% crude protein
[3]Also known as interleukin (IL)-1α
[4]Also known as IL-36
[5]Also known as acidic fibroblast growth factor (a-FGF)
[6]Also known as basic fibroblast growth factor (b-FGF)
[7]Reported in pg/ml
*_P-values_ ≤0.05 determined significant
†_P-values_ 0.05>$P \leq 0.10$ determined a statistical tendency

4.7 Figures

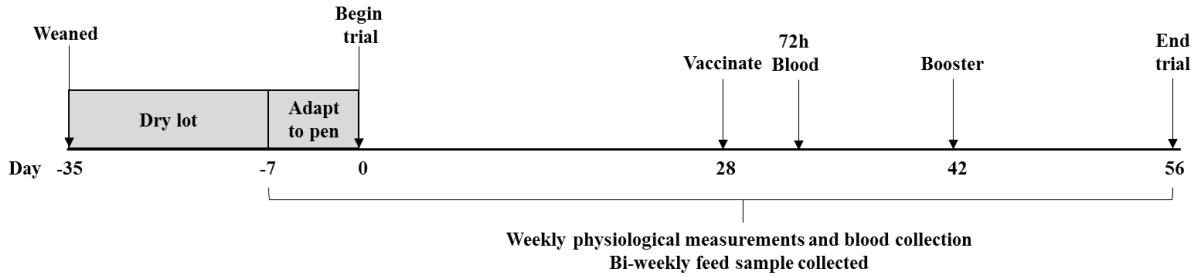


Figure 4.1: Experimental timeline used for steers consuming either endophyte-free (EF) or endophyte-infected (EI) fescue seed with 14% CP (14) or 18% CP (18) from early May to early July 2017.

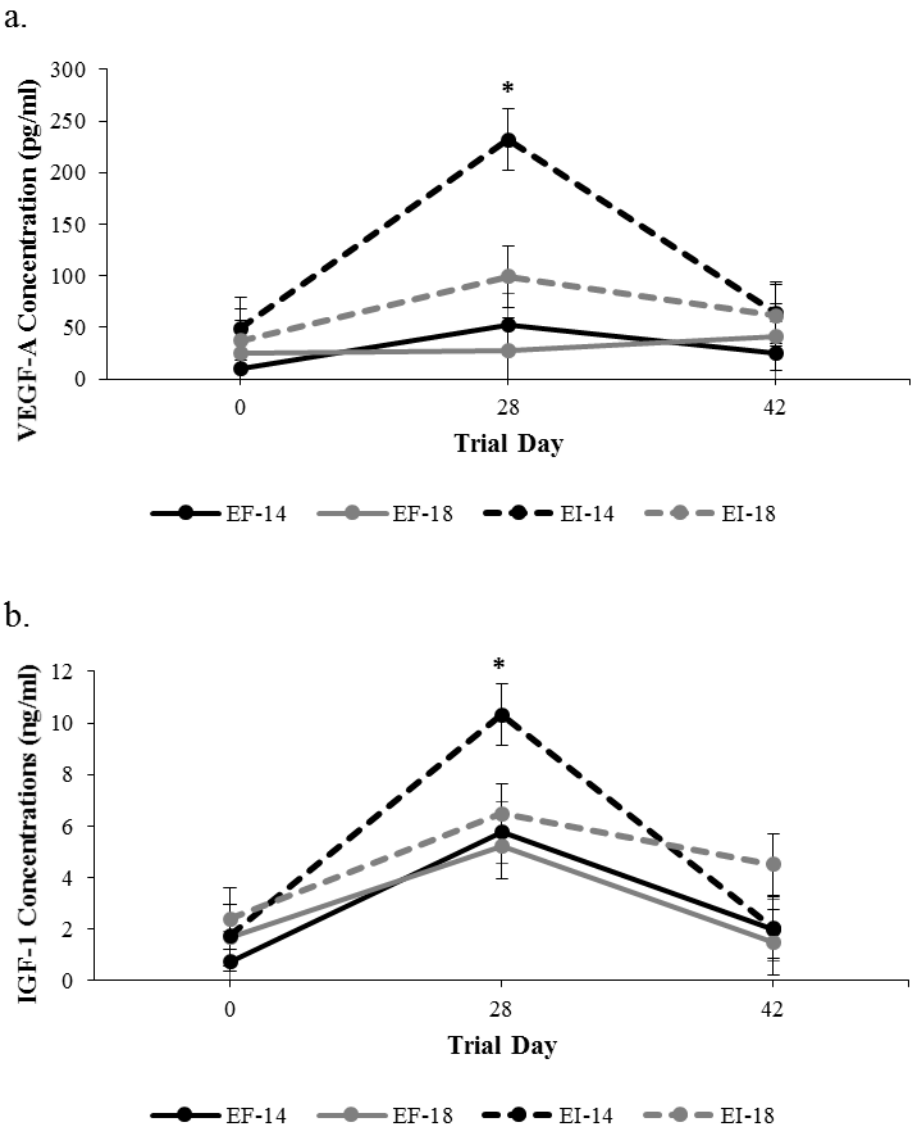


Figure 4.2: Vascular endothelial growth factor-A (VEGF-A) concentrations (a) and insulin-like growth factor (IGF)-1 concentrations (b) in steers consuming endophyte-free fescue seed with 14% CP (solid black line, EF-14; n=8) or with 18% CP (solid gray line, EF-18; n=8) and endophyte-infected fescue seed with 14% CP (dashed black line, EI-14; n=8) or with 18% CP (dashed gray line, EI-18; n=8). **P-values* <0.05 determined significant.

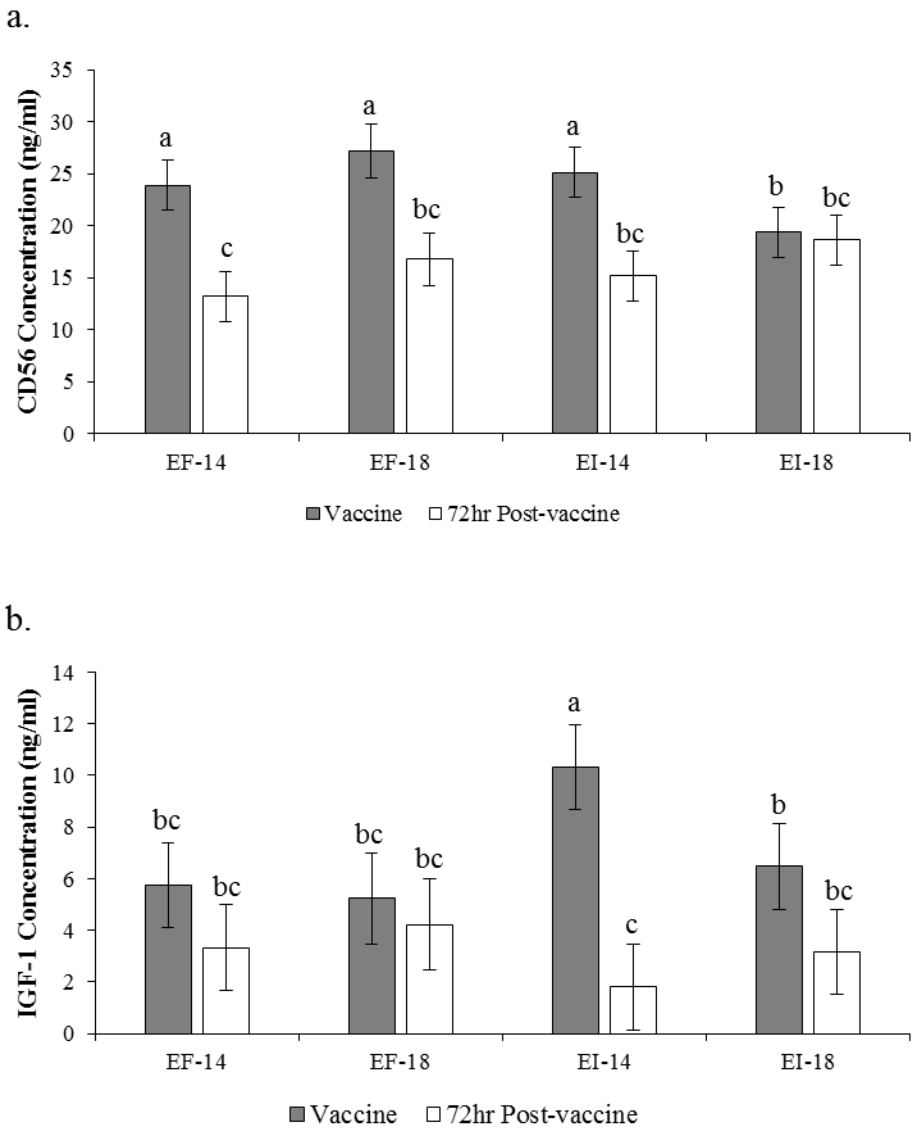


Figure 4.3: Neural-Cell Adhesion Molecule (NCAM or CD56) concentrations (a) and insulin-like growth factor-1 (IGF-1) concentrations (b) in steers consuming either endophyte-free (EF) or endophyte-infected (EI) fescue seed with 14% CP (14) or 18% CP (18) on vaccine day (d28; gray bars) and 72 hr post-vaccination (d31; white bars).

Chapter 5: Effects of protein supplementation and endophyte-infected tall fescue seed on stocker steers: III. Subsequent growth, feedlot performance, and carcass characteristics.

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5.1 Abstract

Performance of stocker steers on endophyte-infected tall fescue is generally very poor and can result in susceptibility to disease in transit to and in the feedlot, thus it is imperative that steers are successfully vaccinated. Recently, it has been shown that supplemental protein can improve performance and immune responsiveness in weaned steers post-vaccination. Therefore, the objective of this study was to evaluate the effect of exposure to endophyte-infected tall fescue and/or supplemental protein at the time of vaccination on subsequent feedlot performance and carcass traits in steers. Thirty-six Angus steers were stratified by weight and treatments were randomly assigned for 56 d: endophyte-free seed and 14% CP (**EF-14**; n=9), endophyte-free seed and 18% CP (**EF-18**; n=9), endophyte-infected seed and 14% CP (**EI-14**; n=9), and endophyte-infected seed and 18% CP (**EI-18**; n=9). Steers were vaccinated on d 28 and given a booster on d 42 of the trial. At the conclusion of the 56 d trial, all steers were placed on an endophyte-infected tall fescue pasture for 255 d and at 16 mo. of age all steers entered the feedlot for a 112 d growing phase followed by a 52 d finishing phase. Body weight was recorded every 28 d during the feedlot period and then steers were harvested and carcass data including HCW, dressing percentage (DP), backfat, ribeye area (REA), % KPH, yield grade (YG), and quality grade (QG) was collected. Data were analyzed using PROC MIXED of SAS. The EI-14 steers had a greater ADG during the growing phase compared to EF-14 steers (1.69 vs. 1.47 ± 0.07 kg/d, respectively; $P<0.05$), however there was no difference when compared to other steer groups. However, no difference was observe in ADG during the finishing phase ($P>0.05$). Steers that received 18% CP had greater

DMI during the growing phase compared to steers that received 14% CP (12.89 vs. 12.33 ± 0.11 kg/d, respectively; $P<0.05$). No differences were observed in HCW, backfat, REA, DP, and QG among treatment groups ($P>0.05$). Overall, previous exposure to endophyte-infected tall fescue did not affect post-harvest carcass characteristics ($P>0.05$). While steers that received 18% CP had greater % KPH (2.3 vs. 1.8 ± 0.2) and YG (3.4 vs. 3.0 ± 0.1) compared to steers that received 14% CP ($P<0.05$). Based on these data, exposure to endophyte-infected tall fescue and/or protein supplementation at the time of vaccination had minimal impact on feedlot performance or carcass quality.

5.2 Introduction

Many calves sold at weaning are generally transported from the Southeast to be backgrounded, otherwise known as a transition period. However, weaning is a major stressful event that potentially results in susceptibility to various diseases (Haley et al., 2005; Lynch et al., 2010). In fact, during this transition period calves will be most susceptible to bovine respiratory disease (BRD; McKinnon and Snodgrass, 2009; Herring, 2014) which can negatively impact subsequent feedlot performance. Preconditioning is a management practice implemented at the time of weaning and includes vaccination against respiratory diseases. However, preconditioning can decrease nutrient intake (Duff and Galyean, 2007), thus lead to compromised performance (Arthington et al., 2013). Fortunately, the addition of protein to the diet results in improved growth performance and immune response in beef steers (Moriel et al., 2015), therefore deceasing the potential risk of developing BRD.

Preconditioning has been shown to be beneficial for the beef industry, specifically feedlot operations by reducing the prevalence of BRD. While 70% of cow-calf operations have implemented vaccination practices, it is less common in small herds (<50 cows; 59%) and in the

Southeast (60%; National Animal Health Monitoring System, 2008). In addition to poor health management practices, a predominant forage in the Southeast is tall fescue (*Lolium arundinaceum* [Schreb.] Darbysh.) which is infected with an endophyte (*Epichloë coenophiala*) that produces toxic ergot alkaloids. Studies have observed that cattle previously grazing on endophyte-infected tall fescue can make compensatory gain in the feedlot (Cole et al., 1987; Lusby et al., 1990; Beconi et al., 1995), however still had reduced carcass weights and quality grade (Lusby et al., 1990). Therefore, the aim of this study was to evaluate the effect of exposure to ergot alkaloids and/or supplemental protein at the time of vaccination on subsequent feedlot performance and carcass traits in steers.

5.3 Materials and Methods

The study was conducted at the Butner Beef Cattle Field Laboratory (BBCFL) in Bahama, NC, and was approved by the Institutional Animal Care and Use Committee at North Carolina State University (17-043-A).

Animals and Experimental Design

The animals and experimental design used in this study have been previously described (Chapter 3). Briefly, steer performance and feed data were collected early May to early July 2017 (d -311 to -255; Figure 5.1). Angus steers (n = 36) approximately 6 mo. of age were weaned, stratified by weight (196.1 ± 3.6 kg), and randomly assigned one of twelve pens (3 steers/pen). Treatments were fed in a total mixed ration (TMR) and designated as follows: endophyte-free seed (EF; control, 0 µg ergovaline/kg of BW) 14% CP (**EF-14**; n = 9), endophyte-free seed 18% CP (**EF-18**; n = 9), endophyte-infected seed (EI; 185 µg ergovaline/kg of BW) 14% CP (**EI-14**; n = 9), and endophyte-infected seed 18% CP (**EI-18**; n = 9). Diets were formulated according to National Research Council (1996) requirements for 1 kg/d ADG at a feed intake of 2.35% of body

weight to prevent refusals. At the conclusion of the 56 d trial, all steers were placed on an endophyte-infected tall fescue pasture for 255 d (i.e. Grazing Period; Figure 5.1). At 16 mo. of age (March 2018), all steers entered the feedlot for a 112 d growing phase followed by a 52 d finishing phase (d 0 to 165; Figure 5.1).

Diet

The growing phase diet consisted of a corn silage TMR made up of 78.9% corn silage, 7.2% ground corn, 12.7% soybean meal, 0.7% limestone, 0.2% trace mineral salt, 0.1% Rumensin90®, and 0.2% Vitamin A, D, and E premix on a DM basis. The first 9 days of the finishing phase, steers were gradually transitioned to the finishing phase diet by simultaneously increasing the concentrate and decreasing the corn silage. The finishing phase diet consisted of a corn silage TMR made up of 15.8% corn silage, 70.6% ground corn, 12.0% soybean meal, 1.25% limestone, 0.2% trace mineral salt, 0.05% Rumensin90®, and 0.1% Vitamin A, D, and E premix on a DM basis. Samples of TMRs were collected every 28 d, composited, and submitted to be analyzed for nutrient content (Cumberland Valley Analytical Services, Waynesboro, PA; Table 5.1).

Feedlot Performance and Carcass Characteristics

At 16 mo. of age, all steers were dewormed with Ivermectin (Durvet Inc.) and Safe-Guard (Merck & Co., Inc.), vaccinated with Bovi Shield Gold 5 (Zoetis Inc.) and Vision 7 (Merck & Co., Inc.), implanted with Revalor-XS (Merck & Co., Inc.) and were placed in the same pen (3 steers/pen) as during 56 d trial. Body weight was recorded every 28 d during this feeding period. All steers were slaughtered at a commercial packing facility (Cargill, Inc.) in September 2018. Hot carcass weight (HCW), back fat thickness, ribeye area (REA), kidney, pelvic, heart (KPH) fat percentage, marbling score, quality grade (QG), and yield grade (YG) were determined by a

qualified USDA grader. Dressing percentage (DP) was calculated using the following equation: % = (carcass weight/live weight) x 100. Percent distribution of QG (Low Choice, Choice, High choice, Prime) within treatment was also evaluated.

Statistical Analysis

Data were analyzed using the MIXED procedure of SAS 9.3 (SAS Inst. Inc., 1996) with repeated measures and pen or animal as the experimental unit, respectively. The model for performance data and carcass characteristics included fescue treatment (EF vs. EI), protein (14% vs. 18%), and interactions. Results were recorded as least squares means ± SEM. Terms with a significance value of $P>0.20$ were removed from the complete model in a stepwise manner to derive the final reduced model for each variable. Pearson correlation coefficient for grazing period ADG and growing phase ADG was analyzed using the CORR procedure of SAS 9.3 (SAS Inst. Inc., 1996). Percent distribution of QG was analyzed by chi-square using the FREG procedure of SAS 9.3 (SAS Inst. Inc., 1996). A statistical significance was reported at a $P\leq0.05$. A tendency was reported at a $P>0.05$ and ≤0.10.

5.4 Results

Growth Performance

All performance data is summarized in Table 5.2. There were no differences in BW between steer groups ($P>0.05$). Interestingly, DMI during the 112 d growing phase was greater for steers fed 18% CP during the treatment period compared to steers fed 14% CP (12.89 vs. 12.33 ± 0.11 kg/d, respectively; $P<0.05$). However, during the 52 d finishing phase the EI-18 steers had a reduction in DMI ($P<0.05$). During the grazing period, ADG did not differ between groups ($P>0.05$). The EI-14 steers had a greater ADG during the 112 d growing phase compared to the

EF-14 group (P<0.05), however not when compared to EF-18 or EI-18 groups. Similarly, the EI-14 steers had a more efficient F:G during the 112 d growing phase compared to the other treatment groups (P<0.05). However, no differences were observe in ADG or F:G during the 52 d finishing phase (P>0.05). A negative linear correlation exists between grazing period ADG and growing period ADG (r = -0.485, P=0.003; Figure 5.2).

Carcass Characteristics

All carcass data is summarized in Table 5.2. At harvest, steers fed 18% CP during the treatment period had greater KPH (2.3 vs. 1.8 ± 0.2 %; P<0.05) and YG (3.4 vs. 3.0 ± 0.1; P<0.05) compared to steers fed 14% CP, respectively. No differences were observed in HCW, backfat, REA, DP, marbling score, and QG between steer groups (P>0.05). When observing the percent distribution of QG, there were no differences observed between steer groups (P>0.05; Table 5.3).

5.5 Discussion

Both vaccination and weaning can elicit a stress-induced immune response, thus calves may experience decreased nutrient intake resulting in compromised growth performance during backgrounding (Arthington et al., 2013). Therefore, one of the most important functions of a backgrounding system is proper management of calves through this stressful adjustment period to ensure sufficient weight gain before feedlot entry. Fortunately, previous research has shown that the addition of protein to the diet during the preconditioning period results in improved immune response and subsequent growth performance in beef steers (Moriel et al., 2015).

Typically, weaned calves from cow-calf operations in the Southeast will be locally backgrounded (e.g. stocker grazing) before being transported a great distance to the feedlot in the Great Plains. It has been suggested that because both cow-calf and stocker operations

predominantly utilize forage, some cow-calf operations can retain calves before selling to a feedlot. The downside is that the predominant forage available in the Southeast is endophyte-infected tall fescue which has adverse effects on cattle performance (Schmidt and Osborn, 1993; Strickland et al., 2009), and thus may negate the value of retaining calves if no other grazing option (i.e. non-toxic tall fescue, other forage variety) is available. Specifically, cattle previously stocker grazing on endophyte-infected tall fescue perform poorly in the feedlot and experience no compensatory gain (Duckett et al., 2001; Scaglia et al., 2013). Contrary to this, numerous studies have observed compensatory gain in the feedlot (Cole et al., 1987; Lusby et al., 1990; Beconi et al., 1995). These inconsistencies could be explained by environmental factors, such as ambient temperature and relative humidity, time of year when cattle were placed in the feedlot, and/or the extreme difference in travel distance to the feedlot. For the current study, EI-14 steers experienced compensatory gain during the 112 d growing phase. While this could be lingering effects from the 56 d trial, EI-14 steers did have a reduced ADG during the grazing period. Even though this reduced ADG was not significant, it is important to note the strong negative correlation between grazing period ADG and growing phase ADG. Simply put, if a steer experienced reduced ADG during the grazing period then that steer would experience improved ADG during the growing phase (i.e. compensatory gain). Moreover, EI-14 steers had better feed efficiency during the growing phase and this would be in agreement with other studies (Beconi et al., 1995; Cole et al., 2001; Duckett et al., 2007).

Carcass traits such as HCW and REA have been negatively impacted when steers grazing endophyte-infected tall fescue during the stocker phase (Lusby et al., 1990; Scaglia et al., 2013). However, other studies have indicated no impact on carcass characteristics (Hancock et al., 1987; Cole et al., 2001; Duckett et al., 2001). These differences could be explained by different environmental conditions, time of year when cattle were placed in the feedlot, and differences in

travel distance to the feedlot. Specifically, Lusby et al. (1990) indicated that heat stress during shipment from pasture to feedlot can exacerbate fescue toxicosis related symptoms which could negatively impact feedlot performance and carcass traits. In the current study, these steers were maintained at the same location for the feedlot phase and therefore did not experience the stress of transportation from pasture to feedlot. Fescue treatment did not have an impact on carcass characteristics, however steers receiving 18% CP during the treatment period had greater percent KPH fat and yield grade. The amount of KPH fat is a subjective measurement assigned by the USDA grader, and expressed as the percentage of HCW with typical ranges from 2.0 to 4.0% (Holland and Loveday, 2013). To evaluate yield grade, these variables are taken into account: amount of external fat, HCW, amount of KPH, and REA then yield grades are rated numerically from 1 to 5 with a yield grade 1 denoting the highest yield (Holland and Loveday, 2013). While there was a significant difference by protein treatment, the greater percent KPH fat and yield grade can most likely be attributed to the increased DMI during the growing phase by these steers.

In summary, in this study neither exposure to ergot alkaloids or supplemental protein at the time of vaccination impacted subsequent feedlot growth performance. Furthermore, carcass characteristics appear to be less affected by exposure to ergot alkaloids or supplemental protein at the time of vaccination, but rather by management and performance of steers in the feedlot.

5.6 Tables

Table 5.1: Nutritional composition of diets.

	Feedlot Phase	
Item	**Growing[1]**	**Finishing[2]**
DM (%)	38.4	70.5
CP (%DM)	11.5	14.8
NDF (%DM)	35.0	12.0
ADF (%DM)	21.8	5.5
TDN (%DM)[3]	72.0	83.7
Ash (%DM)	4.38	4.11
Ca (%DM)	0.50	0.84
P (%DM)	0.34	0.35
Mg (%DM)	0.22	0.15
Na (%DM)	0.12	0.13
K (%DM)	1.17	0.78
Cu (ppm)	20	21
Fe (ppm)	205	131
Mn (ppm)	49	29
Zn (ppm)	59	56

[1]Growing phase (d 0 to 112) TMR composition (DM basis): 90% silage and 10% concentrate
[2]Finishing phase (d113 to 164) TMR composition (DM basis): 34% silage and 66% concentrate
[3]TDN = 92.5135 – (0.7965 x ADF)

Table 5.2: Growth performance during the grazing period and feedlot for growing (d 0 to 112) and finishing (d 113 to 164) phases and carcass characteristics at slaughter (d 165) of steers consuming either endophyte-free (EF) or endophyte-infected (EI) fescue with 14% crude protein (14) or 18% crude protein (18) during a 56 d treatment period[1].

	Treatment[2,3]					*P-value*		
Item	**EF-14**	**EF-18**	**EI-14**	**EI-18**	**SEM**	**Fescue**	**Protein**	**Interaction**
BW, kg								
Trial final[4]	242.1	245.4	242.7	243.9	7.8	0.957	0.776	0.895
d 0	360.2	373.6	349.5	370.1	13.0	0.591	0.200	0.782
d 112	524.4	547.6	538.8	541.5	14.3	0.773	0.370	0.480
d 164	597.5	620.4	607.8	609.1	16.3	0.976	0.465	0.512
ADG, kg/d								
Grazing period[5]	0.46	0.48	0.42	0.52	0.06	0.928	0.184	0.336
d 0 to 112	1.47^{a}	1.55^{ab}	1.69^{b}	1.53^{ab}	0.07	0.159	0.605	0.085†
d 113 to 164	1.37	1.36	1.39	1.32	0.11	0.947	0.708	0.774
DMI, kg/d[6]								
d 0 to 112	12.46^{ab}	13.05^{a}	12.21^{b}	12.73^{a}	0.15	0.068†	0.001*	0.826
d 113 to 164	8.15^{ab}	8.71^{a}	8.52^{a}	7.68^{b}	0.23	0.165	0.551	0.005*
Feed:Gain[7]								
d 0 to 112	2.83^{a}	2.80^{a}	2.41^{b}	2.77^{a}	0.03	<.0001*	<.0001*	<.0001*
d 113 to 164	1.98	2.15	2.14	1.98	0.10	0.960	0.955	0.118
HCW, kg	350.83	363.3	352.2	352.8	10.7	0.674	0.545	0.585
Back fat, cm	1.50	1.57	1.46	1.56	0.08	0.729	0.302	0.835

Table 5.2 (continued).

REA, cm^2	85.8	83.2	87.1	82.6	4.13	0.947	0.117	0.572
KPH, %	1.61^{a}	2.39^{b}	1.94^{ab}	2.22^{b}	0.20	0.680	0.013*	0.221
Yield Grade	3.02^{a}	3.48^{b}	3.01^{a}	3.39^{ab}	0.17	0.764	0.017*	0.815
Marbling[8]	677.8	742.2	726.7	705.6	33.9	0.858	0.527	0.216
Dressing %	62.10	61.95	61.30	61.27	0.53	0.177	0.869	0.910

[a,b] Within row, means without a common superscript significantly differ ($P \leq 0.05$)
[1]Starting at 6 mo. of age, the treatment period lasted 56 d then 8 months following the treatment period, steers entered the feedlot for growing (d 0 to 112) and finishing (d 113 to 164) phases.
[2]Values are reported as least square means for the experiment
[3]EF-14: endophyte-free 14% crude protein; EF-18: endophyte-free 18% crude protein; EI-14: endophyte-infected 14% crude protein; EI-18: endophyte-infected 18% crude protein
[4]Trial final represents the final BW of steers at the completion of the 56 d treatment period.
[5]Grazing period refers to the 255 d period where all steers were placed on an endophyte-infected tall fescue pasture.
[6]Expressed as average DMI/hd/d
[7]Calculated by dividing DMI/hd/d and ADG
[8]Marbling score: modest = 600 to 690; moderate = 700 to 790
*P-*values* <0.05 determined significant
†P-*values* $0.05 > P \leq 0.10$ determined a statistical tendency

Table 5.3: Percent distribution of QG for steers consuming either endophyte-free (EF) or endophyte-infected (EI) fescue with 14% crude protein (14) or 18% crude protein (18) during a 56 d treatment period.

	Treatment				
Quality Grade	**EF-14**	**EF-18**	**EI-14**	**EI-18**	***P-value***
					0.747
Low Choice	11%	11%	11%	22%	
Choice	22%	22%	33%	44%	
High Choice	56%	44%	22%	11%	
Prime	11%	22%	33%	22%	

5.7 Figures

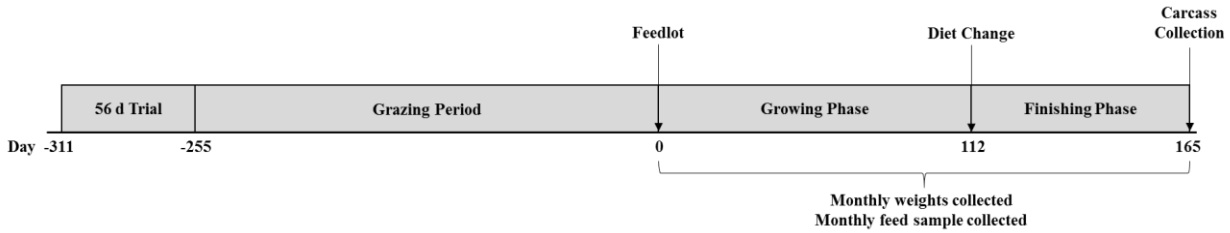

Figure 5.1: Experimental timeline used for steers consuming either endophyte-free (EF) or endophyte-infected (EI) fescue seed with 14% CP (14) or 18% CP (18) from early May to early July 2017 (d -311 to -255), for the grazing period (d -255 to 0), and during the feedlot for growing (d 0 to 112) and finishing (d 113 to 164) phases.

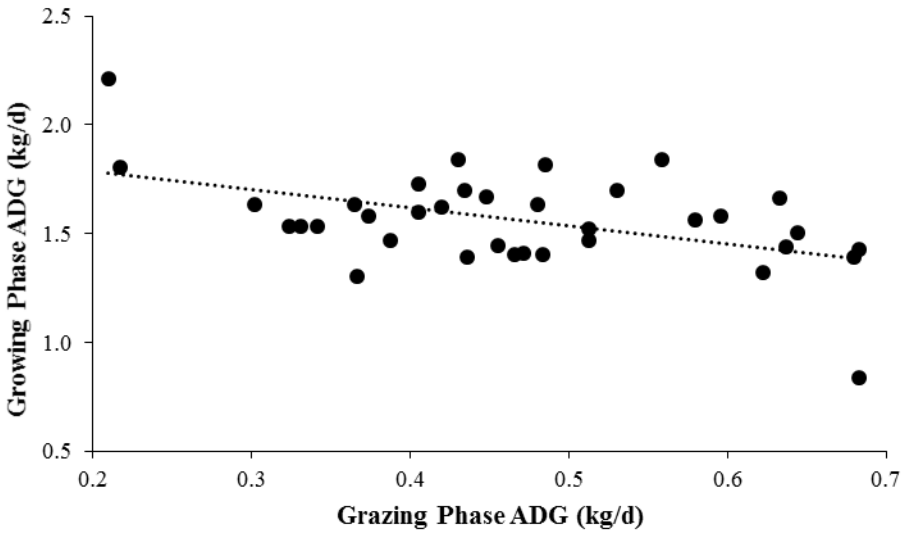

Figure 5.2: Negative linear correlation between grazing period ADG (d -255 to 0) and growing period ADG (d 0 to 112).

Chapter 6: Interpretive Summary and Future Directions

The focus of these studies was to obtain a better understanding of the physiological actions of ergot alkaloids, which is essential to the development of long-lasting solutions to combat losses associated with fescue toxicosis and to improve overall cattle performance. As reported in this dissertation, each sector of the beef industry is either directly or indirectly impacted by fescue toxicosis. Furthermore, the results reported have shed light on multiple areas for future research that would provide valuable information related to the cellular mechanisms of reduced conceptions rates and altered immunity associated with fescue toxicosis. Figure 6.1 displays our current understanding of the direct or indirect mechanisms of fescue toxicosis on reproductive performance and immune responses in cattle.

Many cow-calf operations are located in the Southeast region of the United States mostly because of the abundant forage availability, specifically tall fescue. However, cattle grazing endophyte-infected tall fescue have impaired reproductive performance specifically a reduction in conception rates, which is vital to the success of a cow-calf operation. Additionally, being located in the Southeast high ambient temperatures are common and can induce heat stress conditions. Many symptoms of fescue toxicosis are amplified during periods of heat stress resulting in an inability to maintain a thermoneutral body temperature in grazing cattle. In Chapter 2, the primary focus was an evaluation of the relationship between exposure to ergot alkaloids and heat stress on reproductive performance in beef heifers. Results showed that heifers suffering from heat stress while consuming ergot alkaloids had altered follicular dynamics, reduced estradiol secretion, and all were deemed open 30 days post-insemination. Interestingly, heifers consuming ergot alkaloids that were heat-tolerant (1/8 Senepol breed influence) negated many of the heat stress symptoms associated with fescue toxicosis and therefore helped to improve reproductive performance. This

spurred numerous questions including: 1) Do the mechanisms of ergot alkaloids act in a direct or indirect action to impact reproductive performance? 2) Specifically, do these actions alter bovine follicle (i.e. granulosa) cell function and subsequent follicular dynamics? 3) Or, do these actions alter early embryonic development resulting in reduced pregnancy rates? As a result of this study, two USDA grant proposals have been submitted to address these questions. One major objective in the first USDA grant is to establish the role of ergot alkaloids and/or heat stress on bovine granulosa cell function, hypothesizing that ergot alkaloid exposure will induce apoptosis and upregulate anti-müllerian hormone (AMH) in bovine granulosa cells, thus inhibiting luteinizing hormone (LH) receptor expression and subsequently reduce estradiol secretion. It is believed that the proposed experiments will enhance our understanding of these underlying mechanisms and will ultimately aid in the manipulation of follicle development to ensure proper development of the enclosed oocyte, thus enhancing the likelihood of fertilization. The objective in the second USDA grant is to determine the interaction between direct exposure to ergot alkaloid and heat stress conditions on cleavage rates and early embryonic development in a bovine *in vitro* culture system. It is hypothesized that exposure to ergot alkaloids act adversely to compromise early embryonic development and that the heat stress conditions will exacerbate these adverse effects. It is believed that the proposed experiments will allow us to determine if exposure to a heat stress environment will influence the susceptibility of bovine embryos to the action of ergot alkaloids. Together, these outlined proposals will elucidate the mechanistic role of ergot alkaloids and potentially provide beneficial evidence that incorporation of the slick hair trait will reduce the negative effects of fescue toxicosis on reproductive performance.

With the supply of calves and availability of forage in the Southeast, some cow-calf operations will retain calves after weaning and stocker graze (i.e. background) for 30 to 90 days

before selling to a feedlot. As weaning is a major stressful event, important functions of a backgrounding system are to ensure proper health management of calves and substantial weight gain before feedlot entry, thus ultimately providing a low-stress transition period for calves. However, with endophyte-infected tall fescue being a prominent forage in this region, this could counteract the positive effects of this transition period. Specifically, consuming endophyte-infected tall fescue can lead to a decrease in nutrient intake, thus result in an immuno-compromised animal and would indicate that ergot alkaloids have negative effects on the immune system. In Chapters 3 and 4, the primary focus was evaluating the interaction of ergot alkaloids and supplemental nutrients (i.e. protein) on physiological and immune function of stocker steers. Results showed that supplemental protein may not be beneficial to alleviate physiological symptoms of fescue toxicosis. Moreover, when steers were vaccinated 28 days post-exposure to ergot alkaloids, adaptive immune response (B cell function) was not altered. However, exposure to ergot alkaloids did appear to activate innate immune responses including increased cytokine and chemokine production with protein supplementation providing little to no benefit. To our knowledge, this is the first study to report solely ergot alkaloid-induced activation of innate immunity in beef cattle. These findings warrant additional research to determine the cellular and mechanistic roles ergot alkaloids have on immune cell function. Specifically, it is important to understand the most effective timing of vaccination in regards to ergot alkaloid exposure. For example, if a calf naïve to ergot alkaloids was weaned and vaccinated prior to being placed on endophyte-infected tall fescue for stocker production, would this alter the effectiveness of the vaccine and lead to heightened susceptibility to disease? Additionally, because prolactin plays a critical role in regulation of humoral and cellular immune responses, serum prolactin

concentrations should also be evaluated. Potentially, this could help in clarifying the lack of adaptive immune response observed in Chapter 4.

Finally, while fescue toxicosis has a direct economic impact on cow-calf and stocker operations, there have been numerous claims that cattle from the Southeast perform very poorly during the feedlot phase. This is typically attributed to a weak immune status and cattle will become susceptible to bovine respiratory disease (BRD) which leads to high incidences of morbidity and mortality. One explanation for the poor performance seen in Southeastern cattle would be the previous exposure to ergot alkaloids during the cow-calf and/or stocker phase. In Chapter 5, the primary focus was to evaluate feedlot performance and carcass traits of steers previously exposed to ergot alkaloids. Results showed that while steers exhibited some compensatory gain, carcass traits were not influenced by previous ergot alkaloid exposure. However, one of the limiting factors in this experiment was that steers were finished on-site rather than transported to a feedlot out of the region. Additionally, steers were not transported immediately following the stocker (experimental) phase, and thus the additional 255 days of grazing essentially masked the effects observed during the experimental phase. Therefore, additional research is necessary to elucidate if there is an interaction between previous ergot alkaloid exposure and transportation stress on health/immune status and feedlot performance. As a whole, this work provides novel insights to our understanding of the physiological actions of ergot alkaloids on reproduction and immune function in beef cattle, and provides a strong foundation from which to base future research studies to investigate the cellular and mechanistic role of ergot alkaloids.

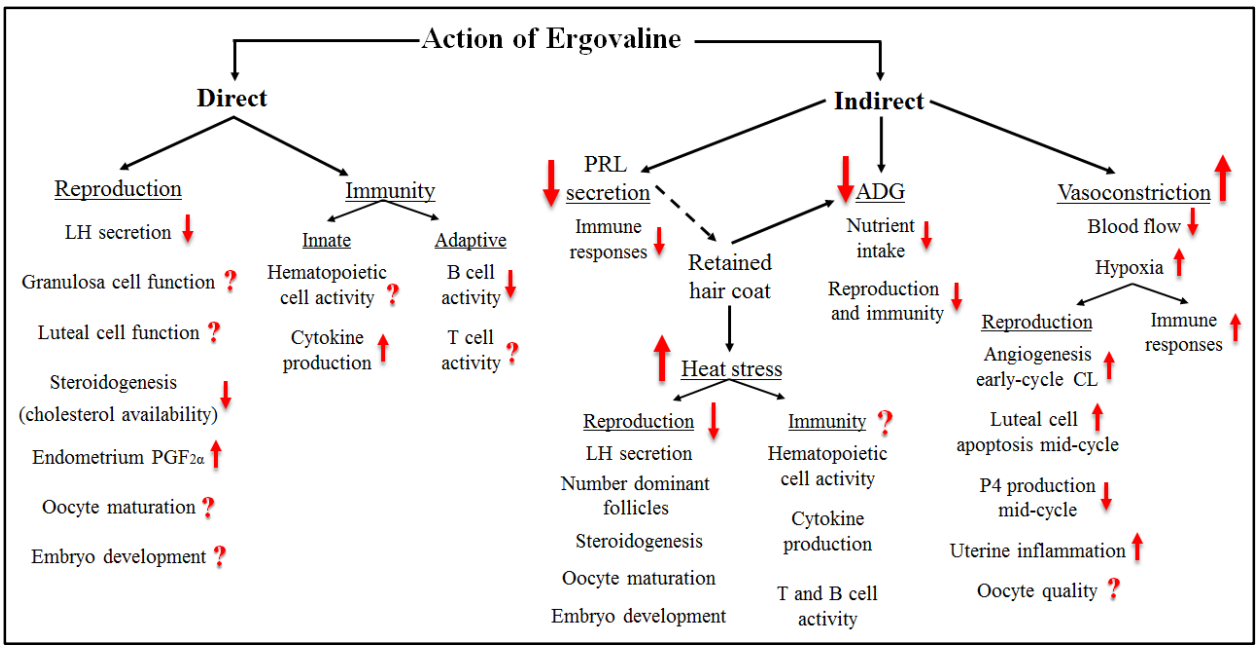

Figure 6.1: Diagram showing the direct or indirect mechanisms of fescue toxicosis on reproductive performance and immune responses in cattle.

References

Adams, G.P., R.L. Matteri, J.P. Kastelic, J.C.H. Ko, and O.J. Ginther. 1992. Association between surges of follicle-stimulating hormone and the emergence of follicular waves in heifers. *J. Reprod. Fertil.* 94:177-188.

Ahmed, N.M., S.P. Schmidt, J.R. Arbona, D.N. Marple, D.I. Bransby, R.L. Carson, D.A. Coleman, and C.H. Rahe. 1990. Corpus luteum function in heifers grazing endophyte-free and endophyte-infected Kentucky-31 tall fescue. *J. Anim. Sci.* 68(Suppl. 1):468.

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.

Aiken, G.E., J.L. Klotz, M.L. Looper, S.F. Tabler, and F.N.Schrick. 2011. Disrupted hair follicle activity in cattle grazing endophyte-infected tall fescue in the summer insulates core body temperatures. *The Professional Animal Scientist* 27(4):336-343. doi:10.15232/S1080-7446(15)30497-6

Aiken, G.E., B.M. Goff, W.W. Witt, I.A. Kagan, B.B. Sleugh, P.L. Burch, and F.N. Schrick. 2012. Steer and Plant Responses to Chemical Suppression of Seedhead Emergence in Toxic Endophyte-Infected Tall Fescue. *Crop Sci.* 52:960-969. doi: 10.2135/cropsci2011.07.0377

Aiken, G.E., J. L. Klotz, J. M. Johnson, J. R. Strickland, and F. N. Schrick. 2013. Postgraze assessment of toxicosis symptoms for steers grazed on toxic endophyte-infected tall fescue pasture. *J. Anim. Sci.* 91(12):5878-5884. doi:10.2527/jas.2012-5964

Akers, R.M., D.E. Bauman, A.V. Capuco, G.T. Goodman, and H.A. Tucker. 1981. Prolactin regulation of milk secretion and biochemical differentiation of mammary epithelial cells in periparturient cows. *Endocrinology*. 109(1):23-30.

Aktas, H., M.B. Wheeler, N.L. First, and M.L. Leibfried-Rutledge. 1995. Maintenance of meiotic arrest by increasing $[cAMP]_i$ may have physiological relevance in bovine oocytes. *J. Reprod. Fertil.* 105:237-245.

Albertini D.F., D. Wickramasinghe, S. Messinger, B.A. Mattson, C.E. Plancha. 1993. Nuclear and Cytoplasmic Changes During Oocyte Maturation. In: Preimplantation Embryo Development. pp. 3-21 Bavister B.D. (eds). Serono Symposia, USA Norwell, Massachusetts. Springer, New York, NY. doi: 10.1007/978-1-4613-9317-7_1

Alberts, B., A. Johnson, J. Lewis, M. Raff, K. Roberts, and P. Walter. 2002a. Chapter 25: Pathogens, Infection, and Innate Immunity. In: Molecular Biology of the Cell, 4th edition. New York: Garland Science.

Alberts, B., A. Johnson, J. Lewis, M. Raff, K. Roberts, and P. Walter. 2002b. Chapter 24: The Adaptive Immune System. In: Molecular Biology of the Cell, 4th edition. New York: Garland Science.

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

Al-Haidary, A., D.E. Spiers, G.E. Rottinghaus, and G.B. Garner. 1995. Effect of administration of ergovaline on the thermoregulatory functions of beef calves under heat stress. *J. Anim. Sci.* 73(Suppl. 1):132.

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

Alila, H.W. and W. Hansel. 1984. Origin of different cell types in the bovine corpus luteum as characterized by specific monoclonal antibodies. *Biol. Reprod.* 31(5):1015-1025.

Allen, V.G., J.P. Fontenot, and D.R. Notter. 1992. Forage systems for beef production from conception to slaughter: II. Stocker systems. *J. Anim. Sci.* 70:588-596.

Allen, V.G., J.P. Fontenot, and F.A. Brock. 2000. Forage systems for production of stocker steers in the upper South. *J. Anim. Sci.* 78:1973-7982.

Arraudeau, M.A. 1989. Breeding strategies for drought resistance. pp. 107–116. In: F.W.C. Baker (ed.) Drought resistance in cereals. CAB International, Wallingford, UK.

Arthington, J.D., R.F. Cooke, T.D. Maddock, D.B. Araujo, P. Moriel, N. Dilorenzo, and G.C. Lamb. 2013. Effects of vaccination on the acute-phase protein response and measures of performance in growing beef calves. *J. Anim. Sci.* 91(4):1831-1837. doi: 10.2527/jas.2012-5724

Artioli, L.F., P. Moriel, M.H. Poore, R.S. Marques, and R.F. Cooke. 2015. Decreasing the frequency of energy supplementation from daily to three times weekly impairs growth and humoral immune response of preconditioning beef steers. *J. Anim. Sci.* 93(11):5430-5441. doi: 10.2527/jas.2015-9457

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

Badinga, L. W. W. Thatcher, T. Diaz, M. Drost, and D. Wolfenson. 1993. Effect of environmental heat stress on follicular development and steroidogenesis in lactating Holstein cows. *Theriogenology*. 39:797-810

Bagley, C.V. 2001. Vaccination Program for Beef Calves. Utah State University. Beef 40.

Baker, T.G., and L.L Franchi. 1967. The fine structure of chromosomes in bovine primordial oocytes. *J. Reprod. Fertil.* 14(3):511-513.

Ball, D.M., C.S. Hoveland, and G.D. Lacefield. 2007. Overview of Southern Forages. Pages 20-25 in: Southern Forages: Modern Concepts for Forage Crop Management 4th edition. IPNI, Norcross, GA.

Barnes, F.L., and W.H. Eyestone. 1990. Early cleavage and the maternal zygotic transition in bovine embryos. *Theriogenology* 33(1):141-152. doi:10.1016/0093-691X(90)90605-S

Barnes, N.M., and T. Sharp. 1999. A review of central 5-HT receptors and their function. *Neuropharmacology* 38:1083-1152. doi:10.1016/S0028-3908 (99)00010-6

Bartlett, S.E., J. Enquist, F.W. Hopf, J.H. Lee, F. Gladher, V. Kharazia, M. Waldhoer, W.S. Mailiard, R. Armstrong, A. Bonci, and J. L. Whistler. 2005. Dopamine responsiveness is regulated by targeted sorting of D2 receptors. *Proc. Natl. Acad. Sci.* 102(32): 11521-11526. doi: 10.1073/pnas.0502418102

Beck, P.A., S.A. Gunter, K.S. Lusby, C.P. West, K.B. Watkins, and D.S. Hubbell, III. 2008. Animal performance and economic comparison of novel and toxic endophyte tall fescues to cool-season annuals. *J. Anim. Sci.* 86(8):2043-2055. doi: 10.2527/jas.2007-0522

Beconi, M.G., M.D. Howard, T.D. Forbes, R.B. Muntigering, N.W. Bradley, and M.J. Ford. 1995. Growth and subsequent feedlot performance of estradiol-implanted vs nonimplanted steers grazing fall-accumulated endophyte-infested or low-endophyte tall fescue. *J. Anim. Sci.* 73(6): 1576-1584. doi:10.2527/1995.7361576x

Ben-Johnathan, N. 1985. Dopamine: a prolactin-inhibiting hormone. *Endocri. Rev. 6:564-589.*

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

Berisha, B., D. Schams, M. Kosmann, W. Amselgruber, and R. Einspanier. 2000. Expression and tissue concentration of vascular endothelial growth factor, its receptors, and localization in the bovine corpus luteum during estrous cycle and pregnancy. *Biol. Reprod.* 63(4):1106-1114.

Bernton, E.W., M.S. Meltzer, and J.W. Holaday. 1988. Suppression of macrophage activation and T-lymphocyte function in hypoprolactinemic mice. *Science* 239: 401-404.

Besser, T.E., C.C. Gay, T.C. McGuire, and J.F. Evermann. 1988a. Transfer of functional immunoglobulin G (IgG) antibody into the gastrointestinal tract accounts for IgG clearance in calves. *J. Virol.* 62(7):2234-2237.

Besser, T.E., C.C. Gay, T.C. McGuire, and J.F. Evermann. 1988b. Passive immunity to bovine rotavirus infection associated with transfer of serum antibody into the intestinal lumen. *J. Virol.* 62(7):2238-2242.

Biron, C.A. 1997. Activation and function of natural killer cell responses during viral infections. *Curr. Opin. Immunol.* 9:24-34.

Bolin, S.R., and J.F. Ridpath. 1990. Range of viral neutralizing activity and molecular specificity of antibodies induced in cattle by inactivated bovine viral diarrhea virus vaccines. *Am. J. Vet. Res.* 51(5):703-707.

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.

Bond, J., J. B. Powell, D. J. Undersander, P. W. Moe, H. F. Tyrrell, and R. R. Oltjen. 1984. Forage composition and growth and physiological characteristics of cattle grazing several varieties of tall fescue during summer conditions. *J. Anim. Sci*. 59:584-593.

Bonilla, F.A., and H.C. Oettgen. 2010. Adaptive Immunity. *J. Allergy Clin. Immunol.* 125(2 Suppl 2):33-40. doi: 10.1016/j.jaci.2009.09.017

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. *J. Agron.* 81:220–223.

Bouton, J.H., R.N. Gates, G.M. Hill, M. Owsley, and D.T. Wood. 1993b. Registration of 'Georgia 5' tall fescue. *Crop Sci.* 33:1045.

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. *Agronomy Journal* 94:567-574.

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

Browning, R., Jr., F.N. Thompson, J.L. Sartin, and M.L. Leite-Browning. 1997. Plasma concentrations of prolactin, growth hormone, and luteinizing hormone in steers administered ergotamine or ergonovine. *J. Anim. Sci.* 75:796-802.

Browning, R., Jr., M.L. Leite-Browing, H.M. Smith, and T. Wakefield, Jr. 1998a. Effect of ergotamine and ergonovine on plasma concentrations of thyroid hormones and cortisol in cattle. *J. Anim. Sci.* 76:1644-1650.

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

Browning, R., Jr. 2004. Effects of endophyte-infected tall fescue on indicators of thermal status and growth in Hereford and Senepol steers. *J. Anim. Sci.* 82:634-643. doi:10.2527/2004.822634x

Bryner, R.W., M. Garcia-Winder, P.E. Lewis, E.K. Inskeep, and R.L. Butcher. 1990. Changes in hormonal profiles during the estrous cycle in old lactating beef cows. *Domest. Anim. Endocrinol.* 7(2):181-189.

Buckner, R.C., J.A. Boling, P.B. Burrus II, L.P. Bush, N. Gay, R.W. Hemken, G.D. Lacefield, and M.R. Siegel. 1985. Agronomic and animal performance of different tall fescue varieties. *Agronomy Notes* 18(1): 1-9.

Buffington, D. E. 1977. Black globe-humidity comfort index for dairy cows. In: Winter meeting of the American Society of Agricultural Engineers, St. Joseph. Trans. Am. Soc. Agric. Eng. pp. 4517.

Burke, J.M., D. E. Spiers, F. N. Kojima, G. A. Perry, B. E. Salfen, S. L. Wood, D. J. Patterson, M. F. Smith, M. C. Lucy, W. G. Jackson, and E. L. Piper. 2001. Interaction of endophyte-infected fescue and heat stress on ovarian function in the beef heifer. *Biol. Reprod.* 65(1):260-268. doi: 10.1095/biolreprod65.1.260

Burke, J.M. and R.W. Rorie. 2002. Changes in ovarian function in mature beef cows grazing endophyte infected tall fescue. *Theriogenology* 57(6):1733-1742. doi: 10.1016/S0093-691X(02)00676-3

Burns, J.C., and D.S. Chamblee. 1979. Adaptation. Chapter 3; pp. 9-30. In: R. Buckner and L. Bush (ed.) Tall Fescue. Agronomy Monographs 20. ASA, CSSA, SSSA, Madison, WI.

Butler, J.E. 1983. Bovine immunoglobulins: an augmented review. *Vet Immunol. Immunopathol.* 4(1-2):43-152.

Cappellozza, B.I., R.F. Cooke, M.M. Reis, P. Moriel, D.H. Keisler, and D.W. Bohnert. 2014. Supplementation based on protein or energy ingredients to beef cattle consuming low-quality cool-season forages: II. Performance, reproductive, and metabolic responses of replacement heifers. *J. Anim. Sci.* 92(6):2725-2734. doi: 10.2527/jas.2013-7442

Caron, M.C., M. Beaulieu, V. Raymond, B. Gange, J. Drouin, J. Lefkowitz, and F. Labrie. 1978. Dopaminergic receptors in the anterior pituitary gland. *J. Biol. Chem.* 253:2244.

Carr, S.B. and D.R. Jacobson. 1969. Bovine physiological responses to toxic fescue and related conditions for application in a bioassay. *J. Dairy Sci.* 52:1792-1799.

Carroll, J.A., J.D. Arthington, C.C. Chase, Jr. 2009. Early waning alters the acute-phase reactions to an endotoxin challenge in beef calves. *J. Anim. Sci.* 87(12): 4167-4172. doi: 10.2527/jas.2009-2016

Carswell, E.A., L.J. Old, R.L. Kassel, S. Green, N. Fiore, and B. Williamson. 1975. An endotoxin-induced serum factor that causes necrosis of tumors. *Proc. Natl. Acad. Sci.* 72(9):3666-3670.

Cartwright, T. C. 1980. Prognosis of Zebu cattle: Research and application. *J. Anim. Sci.* 50:1221-1226.

Cavaillon, J.M. 1994. Cytokines and macrophages. *Biomedicine & Pharmacotheraphy* 48(10):445-453. doi: 10.1016/0753-3322(94)90005-1

Cavaillon, J.M. 2001. Pro- versus anti-inflammatory cytokines: myth or reality. *Cell Mol. Biol.* 47(4):695-702.

Chaplin, D.D. 2010. Overview of the Immune Response. *J. Allergy Clin. Immunol.* 125(Suppl. 2): S3-23. doi: 10.1016/j.jaci.2009.12.980

Chen, L., K.A. Grabowski, J. Xin, J. Coleman, Z. Huang, B. Espiritu, S. Alkan, H.B. Xie, Y. Zhu, F.A. White, J. Clancy, Jr., and H. Huang. 2004. IL-4 Induces Differentiation and Expansion of Th2 Cytokine-Producing Eosinophils. *J. Immunol.* 172:2059-2066. doi:10.4049/jimmunol.172.4.2059

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 coenphialum* Infestation, Bermudagrass, and Nitrogen or Clover on Steers Grazing Tall Fescue Pastures. *J. Prod. Agric.* 4(2):208-213.

Clay, K. 1988. Fungal endophytes of grasses: A defensive mutualism between plants and fungi. *Ecology* 69(1): 10-16. doi: 10.2307/1943155

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

Cole, N.A. 1985. Preconditioning calves for the feedlot. *Vet Clin. North Am. Food Anim. Pract.* 1(2):401-411.

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

Cole, N.A., J.A. Stuedemann, and F.N. Thompson. 2001. Influence of both endophyte infestation in fescue pastures and calf genotype on subsequent feedlot performance of steers. *Professional Animal Scientist* 17: 174-182.

Cooper, J.P., and N.M. Tainton. 1968. Light and temperature requirements for the growth of tropical and temperate grasses. *Herb. Abstr.* 38: 167-176.

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

Cross, M.J., and L. Claesson-Welsh. 2001. FGF and VEFG function in angiogenesis: signaling pathways, biological responses and therapeutic inhibition. *Trends Pharmacol. Sci.* 22(4): 201-207.

Crozet, N. 1984. Ultrastructural aspects of *in vivo* fertilization in the cow. *Gamete Research* 10:241-251.

Cunningham, I. 1948. Tall fescue grass is poison for cattle. *N.Z. J. Agric.* 77:519.

Cunningham, I. 1949. A note on the cause of tall fescue lameness in cattle. *Aust. Vet. J.* 25:27-28. doi: 10.1111/j.1751-0813.1949.tb04752.x

Curtis, S.E. 1983. Environmental Management in Animal Agriculture. pp. 47-96. Iowa State University Press, Ames, IA.

Cusack, P.M., N. McMeniman, and I.J. Lean. 2003. The medicine and epidemiology of bovine respiratory disease in feedlots. *Aust. Vet. J.* 81(8): 480-487.

Dahlman, D.L., M.R. Siegel, and L.P. Bush. 1997. Insecticidal activity of *N*-formylloline. Session 13, p. 5–6.In Proc. XVIII Int. Grasslands Congr. Winnipeg, Canada. 8–19 June 1997.

Daura, M.T., and R.L. Reid. 1991. Energy and protected protein supplements to lambs on endophyte-infected tall fescue pasture. *J. Anim. Sci.* 69:358-368.

Dawe, D.L., J.A. Stuedemann, N.S. Hill, and F. N. Thompson. 1997. Immunosuppression in cattle with fescue toxicosis. Pp. 411-412. In: C.W. Bacon and N.S. Hill (eds.) Neotyphodium/grass interactions. Plenum Press, NY.

Day, M.L., K. Imakawa, M. Garcia-Winder, R.J. Kittok, B.D. Schanbacher, J.E. Kinder. 1986. Influence of prepubertal ovariectomy and estradiol replacement therapy on secretion of luteinizing hormone before and after pubertal age in heifers. *Dom. Anim. Endocrinol.* 3:17-25.

Dikmen, S., E. Alava, E. Pontes, J.M. Fear, B.Y. Dikmen, T.A. Olson, and P.J. Hansen. 2008. Differences in Thermoregulatory Ability between Slick-Haired and Wild-Type Lactating Holstein Cows in Response to Acute Heat Stress. *J. Dairy Sci.* 91(9):3395-3402. doi:10.3168/jds.2008-1072

Dikmen, S., F.A. Khan, H.J. Huson, T.S. Sonstegard, J.I. Moss, G.E. Dahl, and P.J. Hansen. 2014. The *SLICK* hair locus derived from Senepol cattle confers thermotolerance to intensively managed lactating Holstein cows. *J. Dairy Sci.* 97(9):5508-5520. doi:10.3168/jds.2014-8087

Dinarello, C.A. 2000. Proinflammatory Cytokines. *CHEST* 118(2):503-508.

Donovan, G.A., I.R. Dohoo, D.M. Montgomery, and F.L Bennett. 1998. Associations between passive immunity and morbidity and mortality in dairy heifers in Florida, USA. *Prev. Vet Med.* 34(1):31-46.

Donovan, D.C., A.J. Reber, J.D. Gabbard, M. Aceves-Avila, K.L. Galland, K.A. Holbert, L.O. Ely, and D.J. Hurley. 2007. Effect of maternal cells transferred with colostrum on cellular responses to pathogen antigens in neonatal calves. *Am. J. Vet Res.* 68(7):778-782. doi: 10.2460/ajvr.68.7.778

Downey, E.D., R.G. Tait, Jr., M.S. Mayes, C.A. Park, J.F. Ridpath, D.J. Garrick, and J.M. Reecy. 2013. An evaluation of circuiting bovine viral diarrhea virus type 2 maternal antibody level and response to vaccination in Angus calves. *J. Anim. Sci.* 91(9):4440-4450. doi: 10.2527/jas.2012-5890

Downs, S.M. 1993. Factors affecting the resumption of meiotic maturation in mammalian oocytes. *Theriogenology* 39:65-79.

Drewnoski, M.E., E. J. Oliphant, B. T. Marshall, M. H. Poore, J. T. Green, and M. E. Hockett. 2009. Performance of growing cattle grazing stockpiled Jesup tall fescue with varying endophyte status. *J. Anim. Sci.* 87(3):10-34-1041. doi: 10.2527/jas.2008-0977

Drouillard, J.S. 2018. Current situation and future trends for beef production in the United States of America – A review. *Asian-Australa.s J. Anim. Sci.* 31(7): 1007-1016. doi: 10.5713/ajas.18.0428

Duckett, S.K., J.A. Bondurant, J.G. Andrae, J.N. Carter, T.D. Pringle, M.A. McCann, and D.R. Gill. 2001. Effect of grazing tall fescue endophyte types on subsequent feedlot performance and carcass quality. *J. Anim. Sci.* 79(Suppl. 1):221. (Abstract)

Duckett, S.K., R.C. Lacy, J.G. Andrae, C.S. Hoveland, J.H. Bouton, and M.A. McCann. 2007. Animal performance, carcass quality and economics of cattle finished after grazing endophyte-infected, endophyte-free or nonergot alkaloid-producing endophyte-infected tall fescue. *New Zealand Grassland Association: Endophyte Symposium* pp. 253-255.

Duff, G.C., and M.L. Galyean. 2007. BOARD-INVITED REVIEW: Recent advances in management of highly stressed, newly received feedlot cattle. *J. Anim. Sci.* 85(3): 823-840. doi:10.2527/jas.2006-501

Dyer, D.C. 1993. Evidence that ergovaline acts on serotonin receptors. *Life Sciences* 53:223-228. doi:10.1016/0024-3205(93)90555-H

Ealy, A.D., M. Drost, and P.J. Hansen. 1993. Developmental changes in embryonic resistance to adverse effects of maternal heat stress in cows. *J. Dairy Sci.* 76:2899-2905.

Ealy, A.D., S.F. Larson, L. Liu, A.P. Alexenko, G.L. Winkelman, H.M. Kubisch, J.A. Bixby, and R.M. Roberts. 2001. Polymorphic forms of expressed bovine interferon-tau genes: relative transcript abundance during early placental development, promoter sequences of genes and biological activity of protein products. *Endocrinology* 142(7):2906-2915. doi: 10.1210/endo.142.7.8249

Eddy, E.M., J.M. Clark, D. Gong, and B.A. Fenderson. 1981. Origin and migration of primordial germ cells in mammals. *Gamete Research* 4:333-362. doi:10.1002/mrd.1120040407

Edwards, J.L., and P.J. Hansen. 1997. Differential responses of bovine oocytes and preimplantation embryos to heat shock. *Mol. Reprod. Dev.* 46:138-145. doi:10.1002/(SICI)1098-2795(199702)46:2<138::AID-MRD4>3.0.CO;2-R

Eisemann, J. H., G. B. Huntington, M. Williamson, M. Hannah, and M. Poore. 2014. Physiological responses to known intake of ergot alkaloids by steers at environmental temperatures within or greater than their thermoneutral zone. *FCHEM*. 2:96. doi: 10.3389/fchem.2014.00096

El-Banna, A.A., and E.S. Hafez. 1970. Egg transport in beef cattle. *J. Anim. Sci.* 30:430-432.

Elizalde, J.C., J.D. Cremin, Jr., D.B. Faulkner, and N.R. Merchen. 1998. Performance and Digestion by Steers Grazing Tall Fesuce and Supplemented with Energy and Protein. *J. Anim. Sci.* 76:1691-1701.

Elsasser, T.H. and D.J. Bolt. 1987. Dopaminergic-like activity in toxic fescue alters prolactin but not growth hormone or thyroid stimulating hormone in ewes. *Domest. Anim. Endocrinol.* 4(4):259-269.

Elsasser, T.H., R. Fayer, T.S. Rumsey, and G.F. Hartnell. 1994. Recombinant bovine somatotropin blunts plasma tumor necrosis factor-α, cortisol, and thromboxane-B_2 responses to endotoxin *in vivo. Endocrinology* 134: 1082-1088.

Enjalbert, A. and J. Bockaert. 1983. Pharmacological characterization of D2 dopamine receptors negatively coupled with adenylate cyclase in the rate anterior pituitary. *Mol. Pharmacol.* 23:576-584.

Eppig, J.J. 1991. Intercommunication between mammalian oocytes and companion somatic cells. *Bioessays* 13:569-574.

Eppig, J.J. 1996. Coordination of nuclear and cytoplasmic oocyte maturation in eutherian mammals. *Reprod. Fertil. Dev.* 8:485-489.

Essig, H.W., B. Aremu, C.E. Cantrell, M.E. Boyd, and F.T. Withers Jr. 1993. Impacts of Endophyte-Infected Fescue on Cow/Calf Production. *The Professional Animal Scientist.* 9(2):64-69. doi:10.15232/S1080-7446(15)32053-2

Estienne, M.J., K.K. Schillo, B.P. Fitzgerald, S.M. Hielman, N.W. Bradley, and J.A. Boling. 1990. Effects of endophyte-infected fescue on puberty onset and luteal activity in beef heifers. *J. Anim. Sci.* 68(Suppl. 1):468.

Fair, T., P. Hyttel, T. Greve. 1995. Bovine oocyte diameter in relation to maturational competence and transcriptional activity. *Mol. Reprod. Dev.* 42:437-442.

Fanning, M. D., J. C. Spitzer, D. L. Cross, and F. N. Thompson. 1992. A preliminary study of Growth, serum prolactin and reproductive performance of beef heifers grazing Acremonium coenophialum-infected tall fescue. *Theriogenology.* 38(3):375-84.

Farin, C.E., C.L. Moeller, H.R. Sawyer, F. Gamboni, and G.D. Niswender. 1986. Morphometric analysis of cell types in the ovine corpus luteum throughout the estrous cycle. *Biol. Reprod.* 35:1299-1308.

Farin, C.E., C.L. Moeller, H. Mayan, F. Gamboni, H.R. Sawyer, and G.D. Niswender. 1988. Effect of luteinizing hormone and human chorionic gonadotropin on cell populations in the ovine corpus luteum. *Biol. Reprod.* 38:413-421.

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

Filipov, N.M., F.N. Thompson, J.A. Stuedemann, T.H. Elsasser, S. Kahl, R. P. Sharma, C.R. Young, L.H. Stanker, and C.K. Smith. 1999. Increased responsiveness to intravenous lipopolysaccharide challenge in steers grazing endophyte-infected tall fescue compared with steers grazing endophyte-free tall fescue. *J. Endocrinology* 163(2): 213-220.

Finch, A.V. 1986. Body temperature in beef cattle: Its control and relevance to production in the tropics. *J. Anim. Sci.* 62(2):531-542.

Fiserova, A., and M. Pospisil. 1999. Role of ergot alkaloids in the immune system. pp. 451-467. In: Ergot – The genus *Claviceps*, V. Kren and L. Clark (ed.) Harwood, Amsterdam.

Foitzik, K., K. Krause, A.J. Nixon, C.A. Ford, U. Ohnemus, A.J. Pearson, and R. Paus. 2003. Prolactin and its receptor are expressed in murine hair follicle epithelium show hair cycle-dependent expression, and induce catagen. *Am. J. Pathol.* 162(5):1611-1621. doi:10.1016/S0002-9440(10)64295-2

Fortune, J.E., J. Sirois, and S.M. Quirk. 1988. The growth and differentiation of ovarian follicles during the bovine estrous cycle. *Theriogenology* 29:95-109.

Fortune, J.E. and S.M. Quirk. 1988. Regulation of steroidogenesis in bovine preovulatory follicles. *J. Anim. Sci.* 66(suppl. 2):1-9.

Freeman, M.E., B. Kanyicska, A. Lerant, and G. Nagy. 2000. Prolactin: Structure, Function, and Regulation of Secretion. *Physiological Reviews* 80(4):1523-1631.

Friedman, R.M. 1981. Effects of interferons on cell membranes. *Texas Reports on Biology and Medicine* 41:313-316

Fujiwara, T., K. Nakada, H. Shirakawa, and S. Miyazaki. 1993. Development of inositol trisphosphate-induced calcium release mechanism during maturation of hamster oocytes. *Dev. Biol.* 156:69-79.

Fulton, R.W., J.T. Saliki, L.J. Burge, J.M. d'Offay, S.R. Bolin, R.K. Maes, J.C. Baker, and M.L. Frey. 1997. Neutralizing Antibodies to Type 1 and 2 Bovine Viral Diarrhea Viruses: Detection by Inhibition of Viral Cytopathology and Infectivity by Immunoperoxidase Assay. *Clin. Diagn. Lab. Immunol.* 4(3): 380-383.

Fuquay, J.W. 1981. Heat stress as it affects animal production. *J. Anim. Sci.* 52(1):164-174.

Gaffen, S.L., and K. D. Liu. 2004. Overview of interleukin-2 function, production and clinical applications. *Cytokine* 28(3): 109-123. doi: 10.1016/j.cyto.2004.06.010

Galli, C., and G. Lazzari. 1996. Practical aspects of IVM/IVF in cattle. *Animal Reproduction Science* 42:371-379.

Gay, C.C. 1984. The role of colostrum in managing calf health. *Bovine Proc.* 79.

Gerli, R., P. Rambotti, I. Nicoletti, S. Orlandi, G. Migliorati, and C. Riccardi. 1986. Reduced number of natural killer cells in patients with pathological hyperprolactinemia. *Clin. Exp. Immunol.* 64:399-406.

Gilbert, S.F. 2000. Early embryonic development. In: Developmental Biology, 6th edition. Sinauer Associates, Sunderland, MA.

Ginther, O.J., L. Knopf, and J.P. Kastelic. 1989. Temporal associations among ovarian events in cattle during oestrous cycles with two and three follicular waves. *J. Reprod. Fertil.* 87:223-230.

Glass, E.J., R. Baxter, and R.J. Leach, and O.C. Jann. 2012. Genes controlling vaccine response and disease resistance to respiratory viral pathogens in cattle. *Vet. Immunol. Immunopathol.* 148(1-2):90-99. doi: 10.1016/j.vetimm.2011.05.009

Glenn, A.E., C.W. Bacon, R. Price, and R.T. Hanlin. 1996. Molecular phylogeny of *Acremonium* and its taxonomic implications. *Mycologia* 88: 369-383. doi: 10.2307/3760878

Graves, D.T., and Y. Jiang. 1995. Chemokines, a family of chemotactic cytokines. *Crit. Rev. Oral Biol. Med.* 6(2):109-118.

Gray, K.A., T. Smith, C. Maltecca, P. Overton, J.A. Parish, and J.P. Cassady. 2011. Differences in hair coat shedding, and effect on calf weaning weight and BCS among Angus dams. *Livestock Science* 140: 68-71. doi:10.1016/j/livesci.2011.02.009

Griffith, J.W., C.L. Sokol, and A.D. Luster. 2014. Chemokines and Chemokine Receptors: Positioning Cells for Host Defense and Immunity. *Annu. Rev. Immunol.* 32:659-702. doi: 10.1146/annurev-immunol-032713-120145

Guerre, P. 2015. Ergot Alkaloids Produced by Endophytic Fungi of the Genus *Epichloë*. *Toxins* 7: 773-790. doi: 10.3390/toxins7030773

Gunn, D., K.S. Jensen, S. Williams, C. Parsons, T. Hudson, J. England. 2013. Cattle Vaccine Handling and Management Guidelines. University of Idaho. PNW 637.

Gunter, S.A., and P.A. Beck. 2004. Novel endophyte infected tall fescue for growing beef cattle. *J. Anim. Sci.* 82(Suppl.):75-82.

Guretzky, N.A., J.R. Russel, D.R. Strohbehn, and D.G. Morrical. 2005. Grazing and feedlot performance of yearling stocker cattle integrated with spring- and fall-calving beef cows in a year-round grazing system. *J. Anim. Sci.* 83(11):2696-2704. doi: 10.2527/2005.83112696x

Guyton, A.C. 1986. Textbook of Medical Physiology. pp. 849-860. W.B. Saunder Company, Philadelphia, PA.

Hahn, G. L. 1999. Dynamic response of cattle to thermal heat loads. *J. Anim. Sci.* 77: 10-20.

Haley, D.B., D.W. Bailey, and J.M. Stookey. 2005. The effects of weaning beef calves in two stages on their behavior and growth rate. *J. Anim. Sci.* 83(9):2205-2214. doi: 10.2527/2005.8392205x

Hamilton, W.J. and J.A. Laing. 1946. Development of the egg of the cow up to the stage of blastocyst formation. *Journal of Anatomy* 80(4): 194-204.

Hammond, A. C., T. A. Olson, C. C. Chase, Jr., E. J. Bowers, R. D. Randel, C. N. Murphy, D. W. Vogt, and A. Tewolde. 1996. Heat tolerance in two tropically adapted Bos taurus Breeds, Senepol and Romosinuano, compared with Brahman, Angus, and Hereford cattle In Florida. *J. Anim. Sci.* 74:295-303.

Hampl, A., and J.J. Eppig. 1995. Analysis of the mechanism(s) of metaphase I arrest in maturing mouse oocytes. *Development* 121:925-933.

Hancock, D.L., J.E. Williams, H.B. Hedrick, E.E. Beaver, D.K. Larrick, M.R. Ellersieck, G.B. Garner, R.E. Morrow, J.A. Paterson, and J.R. Gerrish. 1987. Performance, body composition, carcass characteristics of steers as influenced by previous forage systems. *J. Anim. Sci.* 65: 1381-1391.

Hannaway, D.B., C. Daly, M.D. Halbleib, D. James, C.P. West, J.J. Volenec, D. Chapman, X. Li, W. Cao, J. Shen, X. Shi, and S. Johnson. 2009. Development of Suitability Maps with Examples for the United States and China. Chapter 3; pp. 33-47. In: H.A. Fribourg, D.B.

Hannaway, and C.P. West (ed.) Tall Fescue for the Twenty-first Century. Agronomy Monographs 53. ASA, CSSA, SSSA, Madison, WI.

Hansel, W. and E.M. Convey. 1983. Physiology of the Estrous Cycle. *J. Anim. Sci.* 57(suppl. 2):404-424.

Hansen, P.J. 2009. Effects of heat stress on mammalian reproduction. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 364(1534):3341-3350. doi: 10.1098/rstb.2009.0131

Hazeleger, N.L., D.J. Hill, R.B. Stubbing, and J.S. Walton. 1995. Relationship of morphology and follicular fluid environment of bovine oocytes to their developmental potential *in vitro. Theriogenology* 43(2):509-522.

Hellstrand, K., and S. Hermodsson. 1987. Role of serotonin in the regulation of human natural killer cell cytotoxicity. *J. Immunol.* 139(3):869-875.

Hellstrand, K, C. Czerkinsky, A. Ricksten, B. Jansson, A. Asea, H. Kylefjord, and S. Hermodsson. 1993. Role of serotonin in the regulation of interferon-gamma production by human natural killer cells. *J. Interferon Res.* 13(1):33-38.

Henricks, D. M., J. F. Dickey and J. R. Hill. 1971. Plasma estrogen and progesterone levels in cows prior to and during estrus. *Endocrinology* 89:1350.

Herring, A.D. 2014. North American beef production. Chapter 5; pp. 132-169. In: L. Kahn and D. Cottle (ed.) Beef Cattle Production and Trade. Csiro Publishing.

Hesse, M. 1981. Alkaloid Chemistry. John Wiley & Sons, Inc., New York.

Higginbotham, G.E., M. Torabi, and J.T. Huber. 1989. Influence of Dietary Protein Concentrations and Degradability on Performance of Lactating Cows during Hot Environmental Temperatures. *J. Dairy Sci.* 72(10): 2554-2564. doi: 10.3168/jds.S0022-0302(89)79396-6

Hill, N.S., W.C. Stringer, G.E. Rottinghaus, D.P. Belesky, W.A. Parrot, and D.D. Pope. 1990. Growth, morphological and chemical component responses of tall fescue to *Acremonium coenophialum. Crop Sci.* 30:156–161.

Hill, N.S., F. N. Thompson, J. A. Stuedemann, D. L. Dawe, and E. E. Hiatt III. 2000. Urinary alkaloid excretion as a diagnostic tool for fescue toxicosis in cattle. *J. Vet Diagn. Invest.* 12:210–217. doi: 10.1177/104063870001200303

Hilton, W.M. 2014. BRD in 2014: where have we been, where are we now, and where do we want to go? *Anim. Health Res. Rev.* 15(2):120-122. doi: 10.1017/S1466252314000115

Hoffmann, B., D. Schams, R. Bopp, M.L. Ender, T. Gimenez, and H. Karg. 1974. Luteotrophic factors in the cow: Evidence for LH rather than prolactin. *J. Reprod. Fert.* 40:77-85.

Holland, R., and D. Loveday. 2013. Understanding yield grades and quality grades for value-added beef producers and marketers. Knoxville, TN: Extension SP755 University of Tennessee Institute of Agriculture.

Holzbauer, M. and K. Racke. 1985. The dopaminergic innervation of the intermediate lobe and of the neural lobe of the pituitary gland. *Med. Biol.* 63:97-116.

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

Hoveland, C.S. 2009. Origin and History. Chapter 1; pp. 3-10. In: H.A. Fribourg, D.B. Hannaway, and C.P. West (ed.) Tall Fescue for the Twenty-first Century. Agronomy Monographs 53. ASA, CSSA, SSSA, Madison, WI.

Hurley, W.L., E.M. Convey, K. Leung, L.A. Edgerton, and R.W. Hemken. 1980. Bovine prolactin, TSH, T and T concentrations as affected by tall fescue summer toxicosis and temperature. *J. Anim. Sci.* 51(2):374-379.

Hyttel, P., T. Greve, and H. Callesen. 1988. Ultrastructure of in-vivo fertilization in superovulated cattle. *J. Reprod. Fertil.* 82(1): 1-13.

IETS. 1998. Manual of the International Embryo Transfer Society, 3rd edition.

Jacobson, D., W. Miller, D. Seath, S. Yates, H. Tookey, and I. Wolff. 1963. Nature of fescue toxicity and progress toward identification of the toxic entity. *J. Dairy Sci.* 46: 416-422. doi: 10.3168/jds.S0022-0302(63)89066-9

Jakubczyk, D. J.Z. Cheng, and S.E. O'Connor. 2014. Biosynthesis of the ergot alkaloids. *Nat. Prod. Rep.*31 (10): 1328-1338. doi: 10.1039/c4np00062e

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

Jones, K.L., S.S. King, K.E. Griswold, D. Cazac, and D.L. Cross. 2003. Domperidone can ameliorate deleterious reproductive effects and reduced weight gain associated with fescue toxicosis in heifers. *J. Anim. Sci.* 81(10):2568-2574. doi: 10.2527/2003.81102568x

Jones, K.L., and S.S. King. 2009. Effect of domperidone supplementation of fescue-fed heifers on plasma and follicular fluid fatty acid composition and oocyte quality. *J. Anim. Sci.* 87:2227-2238. doi:10.2527/jas.2008-1641

Kahl, S., T.H. Elsasser, and J.W. Blum. 1997. Nutritional regulation of plasma tumor necrosis factor-alpha and plasma and urinary nitrite/nitrate responses to endotoxin in cattle. *Proc. Soc. Exp. Biol. Med.* 215(4):370-376.

Kallenbach, R. L., G. J. Bishop-Hurley, M. D. Massie, G. E. Rottinghaus, and C. P. West. 2003. Herbage mass, nutritive value, and ergovaline concentration of stockpiled tall fescue. *Crop Sci.* 43:1001–1005. doi:10.2135/cropsci2003.1001

Kallenbach, R. L. 2015. BILL E. KUNKLE INTERDISCIPLINARY BEEF SYMPOSIUM: Coping with tall fescue toxicosis: Solutions and realities. *J. Anim. Sci.* 93:5487-5495. doi: 10.2527/jas.2015-9229

Kaltenbach, C.C., J.W. Graber, G.D. Niswender, A.V. Nalbandov. 1968. Luteotrophic properties for some pituitary hormones in nonpregnant or pregnant hypophysectomized ewes. *Endocrinology* 82:818-824.

Kawano, H. and S. Daikoku. 1987. Functional topography of the rat hypothalamic dopamine neuron system: retrograde tracing the immunohistochemical study. *J. Comp. Neurol.* 265:242-253.

Kinder, J.E., M. Garcia-Winder, K. Imakawa, M.L. Day, D.D. Zalesky, M.J. D'Occhio, R.J. Kittok, and B.D. Schanbacher. 1983. Influence of different estrogen doses on concentrations of serum LH in acute and chronic ovariectomized cows. *J. Anim. Sci.* 57(suppl. 1):350.

Kirkpatrick, J.G., D.L. Step, M.E. Payton, J.B. Richards, L.F. McTague, J.T. Saliki, A.W. Confer, B.J. Cook, S.H. Ingram, and J.C. Wright. 2008. Effect of age at the time of vaccination on antibody titers and feedlot performance in beef calves. *J. Am. Vet. Med. Assoc.* 233(1):136-142. doi: 10.2460/javma.233.1.136

Kline, J.T., and D. Kline. 1994. Regulation of intracellular calcium in the mouse egg: evidence from inositol triphosphate-induced calcium release, but not calcium-induced calcium release. *Biol. Reprod.* 50:193-203.

Klotz, J.L., L.P. Bush, D.L. Smith, W.D. Shafer, L.L. Smith, B.C. Arrington, and J.R. Strickland. 2007. Ergovaline-induced vasoconstriction in an isolated bovine lateral saphenous vein bioassay. *Journal of Animal Science* 85:2330-2336. doi:10.2527/jas.2006-803

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-naive bovine lateral saphenous veins. *Journal of Animal Science* 86:1021-1028. doi:10.2527/jas.2007-0576

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*. *Journal of Animal* Science 87:2437-2447. doi:10.2527/ jas.2008-1692

Klotz, J.L., B.H. Kirch, G.E. Aiken, L.P. Bush, and J.R. Strickland. 2010. Contractile response of fescue-naive bovine lateral saphenous veins to increasing concentrations of tall fescue alkaloids. *Journal of Animal Science* 88:408-415. doi:10.2527/jas.2009-2243

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. *Journal of Animal Science* 90:682-693. doi:10.2527/jas.2011-4323

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. *Journal of Animal Science* 91:4492-4500. doi:10.2527/jas. 2012-5896

Klotz, J.L., D.H. Kim, A.P. Foote, and D.L. Harmon. 2014. Effects of ergot alkaloid exposure on serotonin receptor mRNA in the smooth muscle of the bovine gastrointestinal tract. *Journal of Animal Science* 92:890-891.

Klotz, J.L., G.E. Aiken, J.R. Bussard, A.P. Foote, D.L. Harmon, B.M. Goff, F.N. Schrick, and J.R. Strickland. 2016. Vasoactivity and Vasoconstriction Changes in Cattle Related to Time off Toxic Endophyte-Infected Tall Fescue. *Toxins* 8(10):271. doi:10.3390/toxins8100271

Koch, G.L. 1990. The endoplasmic reticulum and calcium storage. *Bioessays* 12(11):527-531. doi: 10.1002/bies.950121105

Konishi, M, Y.Aoyagi, T.Takedomi, H.Itakura, T.Itoh, and S.Yazawa. 1996. Presence of granulosa cells during oocyte maturation improved in vitro development of IVM-IVF bovine oocytes that were collected by ultrasound-guided transvaginal aspiration. *Theriogenology* 45(3):573-581. doi:10.1016/0093-691X(95)00404-V

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. doi: 10.2527/jas.2011-4292

Koskinen, M.P. 2012. Alkaloids. Chapter 10; pp. 257-287. In: Asymmetric Synthesis of Natural Products. John Wiley & Sons, Ltd. The Atrium, Southern Gate, Chichester, West Sussex, PO19 8SQ, United Kingdom. doi:10.1002/9781118347300

Kruip, T.A.M., D.G. Cran, T.H. van Beneden, S.J. Dieleman. 1983. Structural changes in bovine oocyte during final maturation *in vivo. Mol. Reprod. Dev.* 8(1):29-47. doi:10.1002/mrd.1120080105

Kubisch, H.M., M.A. Larson, and R.M. Roberts. 1998. Relationship between age of blastocyst formation and interferon-tau secretion by in vitro-derived bovine embryos. *Mol. Reprod. Dev.* 49(3):254-260.

Kuldau, G., and C. Bacon. 2008. Clavicipitaceous endophytes: Their ability to enhance resistance of grasses to multiple stresses. *Biological Control* 46(1): 57-71. doi: 10.1016/j.biocontrol.2008.01.023

Lalman. D., S. Hutson, W. Shearhart, C. Ward, and S. McKinley. 2005. Preconditioning reduces sickness and death loss in weaned calves. *J. Anim. Sci.* 83(Suppl. 2): 21.

Lara-Diaz, V.J., I. Castilla-Cortazar, I. Martin-Estal, M. Garcia-Magarino, G.A. Aguire, J.E. Puche, R.G. de la Garza, L.A. Morales, and U. Munoz. 2017. IGF-1 modulates gene expression of proteins involved in inflammation, cytoskeleton, and liver architecture. *J. Physiol. Biochem.* 73(2): 245-258. doi: 10.1007/s13105-016-0545-x

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(5):1396-1400.

Latch, G.C.M., M.J. Christensen, B.A. Tapper, H.S. Easton, D.E. Hume, and L.R. Fletcher. 2000. Tall fescue endophytes. U.S. Patent 6 111 170. Date issued: 29 August.

Law, A.S., D.N. Logue, T. O'Shea, and T. Webb. 1990. Evidence for a novel factor in steroid free bovine follicular fluid (bFF) which acts to directly suppress follicular development. *Proc SSF* 4(Abstr).

Lee, Y., J.D. Bok, H.J. Lee, H.G. Lee, D. Kim, I. Lee, S.K. Kang, and Y.J. Choi. 2016. Body temperature monitoring using subcutaneously implanted thermologgers from Holstein steers. *AJAS* 29(2):299-306. doi: 10.5713/ajas.15.0353

Leong, D.A., L.S. Frawley, and J.D. Neill. 1983. Neuroendocrine control of prolactin secretion. *Annu. Rev. Physiol.* 45:109-127.

Leuchtmann, A., C.W. Bacon, C.L. Schardi, J.F. White Jr., and M. Tadych. 2014. Nomenclatural realignment of *Neotyphodium* species with genus *Epichloë*. *Mycologia* 106(2): 202-215. doi: 10.3852/13-251

Liebe, D.M. and R.R. White. 2018. Meta-analysis of endophyte-infected tall fescue effects on cattle growth rates. *J. Anim. Sci.* 96:1350-1361. doi: 10.1093/jas/sky055

Littlejohn, M. D., K. M. Henty, K. Tiplady, T. Johnson, C. Harland, T. Lopdell, R. G. Sherlock, W. Li, S. D. Lukefahr, B. C. Shanks, D. J. Garrick, R. G. Snell, R. J. Spelman, and S. R. Davis. 2014. Functionally reciprocal mutations of the prolactin signaling pathway define hairy and slick cattle. *Nat. Commun.* 5:5861. doi:10.1038/ncomms6861

Lledo, P.M., V. Homburger, J. Bockaert and J.D. Vincent. 1992. Differential G Protein-Mediated Coupling of D2 Dopamine Receptors to K^+ and Ca^{2+} Currents in Rat Anterior Pituitary Cells. *Neuron* 8:455-463.

Loius, T.M., H.D. Hafs, D.A. Morrow. 1974. Intrauterine administration of prostaglandin $F_2\alpha$ in cows: progesterone, estrogen, LH, estrus and ovulation. *J. Anim. Sci.* 38(2):347-353.

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. *Journal of Production Agriculture* 3(1): 103-105. doi:10.2134/jpa1990.0103

Lynch, E.M., B. Earley, M. McGee, and S. Doyle. 2010. Characterization of physiological and immunological responses in beef cows to abrupt weaning and subsequent housing. *BMC Vet Res.* 6:37. doi: 10.1186/1746-6148-6-37

Lyons, S. E., A. D. Shaeffer, M. E. Drewnoski, M. H. Poore, and D. H. Poole. 2016. Effect of protein supplementation and forage allowance on the growth and reproduction of beef heifers grazing stockpiled tall fescue. *J. Anim. Sci*. 94:1677–1688. doi:10.2527/jas2015-9969

Lyons, P.C., R.D. Plattner, and C.W. Bacon. 1986. Occurance of peptide and clavinet ergot alkaloids in tall fescue grass. *Science*. 232: 487-489. doi: 10.1126/science.3008328

Mahmood, T., R.S. Ott, G.L. Foley, G.M. Zinn, D.J. Schaeffer, and D.J. Kesler. 1994. Growth and ovarian function of weanling and yearling beef heifers grazing endophyte-infected tall fescue pastures. *Theriogenology* 42:1149-1158.

Malinowski, D.P., and D.P. Belesky. 1999. Infection with leaf fungal endophyte *Neotyphodium coenophialum* increases aluminum sequestration on root surfaces of tall fescue. *J. Plant Nutr.* 22: 1335-1349.

Mariasegaram, M., C. C. Chase, Jr., J. X. Chaparro, T. A. Olson, R. A. Brenneman, and R. P. Niedz. 2007. The slick hair coat locus maps to chromosome 20 in Senepol derived cattle. *Anim. Genet.* 38:54-59. doi:10.1111/j.1365-2052.2007.01560.x

Masui, Y. 1985. Meiotic arrest in animal oocytes. In: Biology of Fertilization, Vol. 1, Pp. 189-219. Metz C.B. and Monroy, A. (eds). San Diego: Academic Press.

Mawhorter, S.D., E. Pearlmean, J.W. Kazura, and W.H. Boom. 1993. Class II major histocompatibility complex molecule expression on murine eosinophils activated in vivo by *Brugia malayi*. *Infect Immun.* 61:5410-5412.

Mayberry, K.J. 2018. Evaluation of Genetic Resistance to Fescue Toxicosis in Purebred Angus Cattle Utilizing Phenotypic Variables, Calf Performance and Cytokine Response. *M.S. Thesis* North Carolina State Univ., Raleigh.

Melbin, J., and D. K. Detweiler. 1993. The cardiovascular system and blood flow. In: Dukes' Physiology of Domestic Animals (eds. Swenson, M. J., Reece, W.O.), Ithaca, NY: Cornell University Press. pp: 64-89.

McBride, W.D., and K. Mathews, Jr. 2011. The Diverse Structure and Organization of U.S. Beef Cow-Calf Farms. Washington DC: U.S. Department of Agriculture.

McClanahan, L.K., G.E. Aiken, and C.T. Dougherty. 2008. Case study: Influence of rough hair coats and steroid implants on the performance and physiology of steers grazing endophyte-infected tall fescue in the summer. *The Professional Animal Scientist* 24:269-276.

McCollough, S.F., E.L. Piper, A.S. Moubarak, Z.B. Johnson, R.J. Petroski, and M. Flieger. 1994. Effect of tall fescue ergot alkaloids on peripheral blood flow and serum prolactin in steers. *J. Anim. Sci.* 72(Suppl. 1):144.

McCracken, J.A., E.E. Custer, and J.C. Lamsa. 1999. Luteolysis: a neuroendocrine-mediated event. *Physiol. Rev.* 79(2):263-323. doi: 10.1152/physrev.1999.79.2.263

McKenzie, P.P., and B.H. Erickson. 1989. The effects of fungal-infected fescue on hormonal secretion and ovarian development in the beef heifer. *J. Anim. Sci.* 67(suppl. 2):58.

McKenzie, P.P., and B.H. Erickson. 1991. Effects of fungal-infested fescue on gonadotrophin secretion and folliculogenesis in beef heifers. *J. Anim. Sci.* 69(suppl. 1):387.

McKinnon, B.R., and H.S. Snodgrass. 2009. Getting Started in the Cattle Business in Virginia. *Virginia Cooperative Extension Publications* 400-790.

McLaren, A. 1988. The developmental history of female germ cells in mammals. *Oxford Rev. Reprod. Biol.* 10:162-179.

McNatty K. P., D. M. Smith, A. Makris, R. Osathanondh, and K. J. Ryan. 1979. The microenvironment of the human antral follicle: interrelationships among the steroid levels in antral fluid, the population of granulosa cells, and the status of the oocyte in vivo and in vitro. *J. Clin. Endocrinol. Metab.* 49(6):851-860.

McPherson, G.A., P.M. Beart. 1983. The selectivity of some ergot derivatives for alpha 1 and alpha 2-adrenoceptors of rat cerebral cortex. *European Journal of Pharmacology* 91:363-369. doi:10.1016/0014-2999(83) 90159-0

Mercadante V. R., P. L. Fontes, F. M. Ciriaco, D. D. Henry, P. Moriel, A. D. Ealy, S. E. Johnson, N. DiLorenzo, and G. C. Lamb. 2016. Effects of recombinant bovine somatotropin administration at breeding on cow, conceptus, and subsequent offspring performance of beef cattle. *J. Anim. Sci.* 94(5):2128-2138. doi: 10.2527/jas.2015-0217

Morgan, D.A., F.W. Ruscetti, and R. Gallo. 1976. Selective in vitro growth of T lymphocytes from normal human bone marrow. *Science* 193: 1007-1008.

Morgan-Jones, G., and W. Gams. 1982. Note on Hyphomycetes. XLI. An endophyte of *Festuca arundinacea* and the anamorph of *Epichloë typhina*, new taxa in one of two new sections of *Acremonium. Mycotaxon* 15: 311-318.

Moriel. P., L.F.A. Artioli, M.H. Poore, A.W. Confer, R.S. Marques, and R. F. Cooke. 2015. Increasing the metabloizable protein supply enhanced growth performance and led to

variable results on innate and humoral immune response of preconditioning beef steers. *J. Anim. Sci.* 93(9):4473-4485. doi:10.2527/jas.2015-9238

Murdoch, W.J., R.A. Dailey, and E.K. Inskeep. 1981. Preovulatory changes in prostaglandins E_2 and $F_2\alpha$ in ovine follicles. *J. Anim. Sci.* 53(1):192-205.

Murphy, M.G., W.J. Enright, M.A. Crowe, K. McConnell, L.J. Spicer, M.P. Boland, and J.F. Roche. 1991. Effect of dietary intake on pattern of growth of dominant follicles during the oestrous cycle in beef heifers. *J. Reprod. Fertil.* 92(2):333-338.

Murray, R.Z., J.G. Kay, D.G. Sangermani, and J.L. Stow. 2005. A role for the phagosome in cytokine secretion. *Science* 310(5753):1492-1495. doi: 10.1126/science.1120225

Nasr, H. and O.H. Pearson. 1975. Inhibition of prolactin secretion by ergot alkaloids. *Acta Endocrinol* 80:429-443. doi: 10.1530/acta.0.0800429

National Animal Health Monitoring System. 2008. Part IV: Reference of Beef Cow-calf Management Practices in the United States, 2007-08. *USDA, Animal and Plant Health Inspection Service, National Animal Health Monitoring System.*

National Research Council. 1996. Nutrient Requirements of beef cattle, 7th Rev. Edn. Washington, DC: National Academy Press.

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.

Nishimura, R. and K. Okuda. 2010. Hypoxia is important for establishing vascularization during corpus luteum formation in cattle. *J. Repro. Dev.* 56:110-116. doi:10.1262/jrd.09-162E.

Niswender G. D., R. H. Schwall, T. A. Fitz, C. E. Farin, and H. R. Sawyer. 1985. Regulation of luteal function in domestic ruminants: new concepts. *Recent Prog. Horm. Res.* 41:101-151.

Niu, M., Y. Ying, P. A. Bartell, and K. J. Harvatine. 2014. The effects of feeding time on milk production, total-tract digestibility, and daily rhythms of feeding behavior and plasma metabolites and hormones in dairy cows. *J. Dairy Sci.* 97:7764–7776. doi:10.3168/jds.2014-8261

Nixon, A.J., C.A. Ford, J.E. Wildermoth, A.J. Craven, M.G. Ashby, and A.J. Pearson. 2002. Regulation of prolactin receptor expression in ovine skin in relation to circulating prolactin and wool follicle growth status. *J. Endocrinol.* 172(3):605-614.

Nordenson, N. 2006. Gale Encyclopedia of Medicine. 3rd ed. Gale Group, Detroit, MI.

Northey, D.L. and L.R. French. 1980. Effect of embryo removal and intrauteri infusion of embryonic homogenates on the lifespan of the bovine corpus luteum. *J. Anim. Sci.* 298-302.

O'Connor, J. C., R. H. McCusker, K. Strle, R. W. Johnson, R. Dantzer, and K. W. Kelley. 2008. Regulation of IGF-1 function by proinflammatory cytokines: At the interface of immunology and endocrinology. *Cell. Immunol.* 252:91–110. doi:10.1016/j. cellimm.2007.09.010

Odde, K.G., H.S. Ward, G.H. Kiracofe, R.M. McKee, and R.J. Kittok. 1980. Short estrous cycles and associated serum progesterone levels in beef cows. *Theriogenology* 14:105-112.

Oliver, J.W. 1997. Physiological manifestations of endophyte toxicosis in ruminant and laboratory species. pp. 311-346. In: C.W. Bacon and N.S. Hill (ed.) Neotyphodium/grass interactions. Springer, Boston, MA.

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

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

Olson, T. A., C. Lucena, C. C. Chase, and A. C. Hammond. 2003. Evidence of a major gene influencing hair length and heat tolerance in *Bos taurus* cattle. *J. Anim. Sci.* 81:80-90. doi:10.2527/2003.81180x

Opal, S.M., and V.A. DePalo. 2000. Anti-Inflammatory Cytokines. *CHEST* 117(4):1162-1172.

Osborn, T.G. 1988. Effect of consuming fungus-infected and fungus-free tall fescue and ergotamine tartrate on certain physiological variables of cattle in environmentally-controlled conditions. M.S. Thesis. Auburn University, AL.

O'Shea, J.D. 1987. Heterogeneous cell types in the corpus luteum of sheep, goats, and cattle. *J. Repro. Fertil.* 34(Suppl.):71.

O'Shea, J.J., D.M. Frucht, and C.S. Duckett. 2019. Cytokines and cytokines receptors. pp. 12.1-12.22. In: R. Rich, T.A. Fleisher, W.T. Shearer, B.L. Kotzin, H.W. Schroeder Jr. (eds.) Clinical immunology: principles and practice (2^{nd} ed.), Mosby, St. Louis, MO.

Parameswaran, N., and S. Patial. 2010. Tumor Necrosis Factor-α Signaling in Macrophages. *Crit. Rev. Eukaryot. Gene Expr.* 20(2):87-103.

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(11):2856-2868. doi: 10.2527/2003.81112856x

Parrish, J.J., J.L. Susko-Parrish, M.L. Leibfried-Rutledge, E.S. Critser, W.H. Eyestone, and N.L. First. 1986. Bovine *in vitro* fertilization with frozen-thawed semen. *Theriogenology* 25(4):591-600.

Pate, J.L., W.A. Condon. 1982. Effects of serum and lipoproteins on steroidogenesis in cultured bovine luteal cells. *Molecular and Cellular Endocrinology.* 28(3):551-62.

Pate, J.L., W.A. Condon, 1989. Regulation of steroidogenesis and cholesterol synthesis by prostaglandin F-2 alpha and lipoproteins in bovine luteal cells. *J. Reprod. Fertil.* 87(2):439-46.

Patel, O.V., I. Domeki, N. Sasaki, T. Takahashi, M. Hirako, R.G. Sasser, and P. Humblot. 1995. Effect of fetal mass, number and stage of gestation on pregnancy-specific protein B concentrations in the bovine. *Theriogenology* 44(6):827-833. doi:10.1016/0093-691X(95)00268-D

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

Pedersen, J.F., R.L. Haaland, C.S. Hoveland, C.D. Berry, S.P. Schmidt, and R.R. Harris. 1983. Registration of Au Triumph Tall Fescue. *Crop Sci.* 23(1): 182. doi:10.2135/cropsci1983.00-11183X002300010062x

Pedersen J.F., G. Lacefield, D. Ball. 1990. A review of the agronomic characteristics of endophyte-free and endophyte-infected tall fescue. *Appl. Agric. Res.* 5:188–194.

Pertz, H.H., E. Eich. 1999. Ergot alkaloids and their derivatives as ligands for serotoninergic, dopaminergic, and adrenergic receptors. pp. 441-450. In: V. Kren, L. Cvak (ed.) Ergot. Harwood Academic Publishers, Amsterdam, The Netherlands.

Poole, D.H., S.E. Lyons, R.K. Poole, and M.H. Poore. 2018. Ergot alkaloids induce vasoconstriction of bovine uterine and ovarian blood vessels. *J. Anim. Sci.* 96(11): 4812-4822. doi: 10.1093/jas/sky328

Porter, J.K., C.W. Bacon, and J.D. Robbins. 1979. Lysergic acid amide derivatives from *Balansia Epichloë* and *Balansia Claviceps* (*Clavicipitaceae*). *J. Nat. Prod.* 42(3): 309-314.

Porter, J.K., and F.N. Thompson, Jr. 1992. Effect of Fescue Toxicosis on Reproduction in Livestock. *J. Anim. Sci.* 70:1594-1603.

Porto-Neto, L. R., D. M. Bickhart, A. J. Landaeta-Hernandez, Y. T. Utsunomiya, M. Pagan, E. Jimenez, P. J. Hansen, S. Dikmen, S. G. Schroeder, E. Kim, J. Sun, E. Crespo, N. Amati, J. B. Cole, D. J. Null, J. F. Garcia, A. Reverter, W. Barendse, and T. S. Sonstegard. 2018. Convergent Evolution of Slick Coat in Cattle through Truncation Mutations in the Prolactin Receptor. *Frontiers in Genetics* 9: 57. doi: 10.3389/fgene.2018.00057

Price, E.O., J.E. Harris, R.E. Borgwardt, M.L. Sween, and J.M. Connor. 2003. Fenceline contact of beef calves with their dams at weaning reduces the negative effects of separation on behavior and growth rate. *J. Anim. Sci.* 81:116-121.

Pritchard, R.H. and J.K. Mendez. 1990. Effects of preconditioning on pre- and post-shipment performance of feeder steers. *J. Anim. Sci.* 68:28-34.

Rajakoski, E. 1960. The ovarian follicular system in sexually mature heifers with special reference to seasonal, cyclical and left-right variations. *Acta. Endocrinol.* 34(suppl. 52):1-68.

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.

Reynolds, L.P., A.T. Grazul-Bilska, and D.A. Redmer. 2000. Angiogenesis in the corpus luteum. *Endocrine* 12: 1-9. doi: 10.1385/ENDO:12:1:1

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.

Ricci, A., S. Marietta, S. Greco, and A. Bisetti. 1997. Expression of dopamine receptors in immune organs and circulating immune cells. *Clin. Exp. Hypertens* 19:59-71.

Rice, R.L., D.J. Blodgett, G.G. Schurig, W.S. Swecker, J.P. Fontenot, V.G. Allen, and R.M. Akers. 1997. Evaluation of humoral immune response in cattle grazing endophyte-infected or endophyte-free fescue. *Vet Immunol. Immunopathol.* 59:285-291.

Richards, J.S. 1994. Hormonal control of gene expression in the ovary. *Endocr. Rev.* 15:725-751.

Richeson, J.T., P.A. Beck, M.S. Gadberry, S.A. Gunter, T.W. Hess, D.S. Hubbell, and C. Jones. 2008. Effects of on-arrival versus delayed modified-live virus vaccination on health, performance, and serum infectious bovine rhinotracheitis titers of newly-received beef calves. *J. Anim. Sci.* 86:999-1005.

Roberts, C.A., R.L. Kallenbach, N.S. Hill, G.E. Rottinghaus, and T.J. Evans. 2009. Ergot alkaloid concentrations in tall fescue hay during production and storage. *Crop Sci.* 49:1496-1502. doi: 10.2135/cropsci2008.08.0453

Roeber, D.L., N.C. Speer, J.G. Gentry, J.D. Tatum, C.D. Smith, J.C. Whittier, G.F. Jones, K.E. Belk, and G.C. Smith. 2001. Feeder cattle health management: Effects on morbidity rates, feedlot performance, arcass characteristics, and beef palatability. *The Professional Animal Scientist* 17:39-44.

Rosenbaum, M.J., E.A. Edwards, and E.V. Sullivan. 1970. Micromethods for respiratory virus sero-epidemiology. Health Lab. Sci. 7:42-52.

Roth, Z., R. Meidan, A. Shaham-Albalancy, R. Braw-Tal, D. Wolfenson. 2001. Delayed effect of heat stress on steroid production in medium-sized and preovulatory bovine follicles. *Reproduction* 121(5):745-751.

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

Rottinghaus, G. E., L. M. Schultz, P. F. Ross, and N. S. Hill. 1993. An HPLC method for the Detection of ergot in ground and pelleted feeds. *J. Vet. Diagn. Investig*. 5:242-247. doi:10.1177/104063879300500216

Rowson, L.E., H.R. Tervit, A. Brand. 1972. The use of prostaglandins for synchronization of oestrus in cattle. *J. Reprod. Fertil.* 29(1):145.

Saameli. K. 1978. Effects on the uterus. In: Ergot Alkaloids and Related Compounds, Vol. 49, pp. 233-319. Handbook of Experimental Pharmacology. B. Berde and H.O. Schild. (ed.) Springer, New York, NY.

Saker, K.E., V.G. Allen, J. Kalnitsky, C.D. Thatcher, W.S. Sweckeer Jr., and J.P. Fontenot. 1998. Monocyte immune cell response and copper status in beef steers that grazed endophyte-infected tall fescue. *J. Anim. Sci.* 76(10):2694-27000.

Samford-Grigsby, M.D., B.T. Larson, J.C. Forcherio, D.M. Lucas, J.A. Paterson, and M.S. Kerley. 1997. Injection of a Dopamine Antagonist into Holstein Steers to Relieve Symptoms of Fescue Toxicosis. *J. Anim. Sci.* 75:1026-1031.

SAS. SAS System for Mixed Models; SAS Inst. Inc.: Cary, NC, USA, 1996.

Sasser, R.G., C.A. Ruder, K.A. Ivani, J.E. Butler, and W.C. Hamilton. 1986. Detection of pregnancy by radioimmunoassay of a novel pregnancy-specific protein in serum of cows and a profile of serum concentrations during gestation. *Biol. Reprod.* 35(4):936-942.

Scaglia, G., J.P. Fontenot, W.S. Swecker Jr., B.A. Corl, H.T. Boland, A.O. Abaye, and R.S. Smith. 2013. Effect of forage type in the stocker phase and its effect on subsequent feedlot performance and carcass characteristics of beef steers. *The Professional Animal Scientist* 29(2): 133-140. doi:10.15232/S1080-7446(15)30211-4

Schams, D. and V. Reinhardt. 1974. Influence of the season on plasma prolactin levels in cattle from birth to maturity. *Hormone Res.* 5:217.

Schams, D. W. Amselgruber, R. Einspanier, F. Sinowatz, and D. Gospodarowicz. 1994. Localization and tissue concentration of basic fibroblast growth factor in the bovine corpus luteum. *Endocrine* 2:907-912.

Schardl, C.L. 1996. *Epichloë* species: fungal symbionts of grasses. *Annu. Rev. Phytopathol.* 34: 109-130. doi: 10.1146/annurev.phyto.34.1.109

Schiff, P.L., Jr. 2006. Ergot and Its Alkaloids. *Am. J. Pham. Educ.* 70(5): 98. doi: 10.5688/aj700598

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.

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

Schuenemann, G.M., J.L. Edwards, F.M. Hopkins, N.R. Rohrbach, H.S. Adair, F.N. Scenna, J.C. Waller, J.W. Oliver, A.M. Saxton, and F.N. Schrick. 2005a. Fertility aspects in yearling beef bulls grazing endophyte-infected tall fescue pastures. *Reprod. Fertil. Dev.* 17(4):479-486.

Schuenemann, G.M., J.L. Edwards, M.D. Davis, H.E. Blackmon, F.N. Scenna, N.R. Rohrbach, A.M. Saxton, H.S. Adair, F.M. Hopkins, J.C. Waller, and F.N. Schrick. 2005b. Effects of administration of ergotamine tartrate on fertility of yearling beef bulls. *Theriogenology* 63(5):1407-1418. doi:10.1016/j.theriogenology.2004.07.014

Schuenemann, G.M., M.E. Hockett, J.L. Edwards, N.R. Tohrbach, K.F. Breuel, and F.N. Schrick. 2005c. Embryo development and survival in beef cattle administered ergotamine tartrate to simulate fesecue toxicosis. *Reproductive Biology* 5(2):137-150.

Schultz, R.M., and G.S. Kopf. 1995. Molecular Basis of Mammalian Egg Activation. *Current Topics in Developmental Biology* 30:21-62. doi:10.1016/S0070-2153(08)60563-3

Schultze, A.E., B.W. Rohrbach, H.A. Fribourg, J.C. Waller, and J.W. Oliver. 1999. Alterations in bovine serum biochemistry profiles associated with prolonged consumption of endophyte infected tall fescue. *Vet. Hum. Toxicol.* 41:133-139.

Seals, R. C., G. M. Schuenemann, J. W. Lemaster, A. M. Saxton, J. C. Waller, and F. N. Schrick. 2005. Follicular dynamics in beef heifers consuming ergotamine tartrate as a model of endophyte-infected tall fescue consumption. *JAVA.* 4:97-102.

Shemesh, M., N. Ayalon, and H.R. Lindner. 1968. Early effect of conceptus on plasma progesterone level in the cow. *J. Reprod. Fertil.* 15:161-164.

Sibley, D.R. and F.J. Monsma Jr. 1992. Molecular biology of dopamine receptors. *Trends Pharmacol. Sci.* 13(2):61-69.

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. 1984. A fungal endophyte in tall fescue: incidence and dissemination. *Phytopathology* 74(8): 932-937.

Siegel, M.R., G.C.M. Latch, and M.C. Johnson. 1985. *Acremonium* fungal endophytes of tall fescue and perennial ryegrass: significance and control. *Plant Dis.* 69:179-181.

Siegel, M.R., and L.P. Bush. 1996. Defensive chemicals in grass-fungal endophyte associations. *Recent Adv. Phytochem*. 30:82-119.

Silva, G.M., M.H. Poore, J. Ranches, and P. Moriel. 2018. Effects of timing of vaccination relative to weaning and post-weaning frequency of energy supplementation on growth and immunity of beef calves. *J. Anim. Sci.* 96(1):318-330. doi: 10.1093/jas/skx021

Sirard, M.A., and S. Bilodeau. 1990. Granulosa cells inhibit the resumption of meiosis in bovine oocytes *in vitro. Biol. Reprod.* 43(5):777-783.

Sleper, D.A., and C.P. West. 1996. Tall Fescue. Chapter 15; pp. 471-502. In: L.E. Moser, D.R. Buxton, and M.D. Casler (ed.) Cool-Season Forage Grasses. Agron. Monogr. 34. ASA, CSSA, SSSA, Madison, WI.

Smejkal-Jagar, L., and M. Boranic. 1994. Serotonin, serotoninergic agents and their antagonists suppress humoral immune reaction in vitro. *Res. Exp. Med. Berl.* 194:297-304.

Smith, E.L., and A. Holm. 1948. The transfer of immunity to the new-born calf from colostrum. *J. Biol. Chem.* 175(1):349-357.

Smith, M. F., E. W. McIntush, and G. W. Smith. 1994. Mechanisms associated with corpus luteum development. *J. Anim. Sci.* 72(7):1857-1872.

Smith, T., and J.P. Cassady. 2015. BILL E. KUNKLE INTERDISCIPLINARY BEEF SYMPOSIUM: Genetic resistance to the effects of grazing endophyte-infected tall fescue. *J. Anim. Sci.* 93: 5506-5511. doi: 10.2527/jas2015-9285

Smith, W.L., N. Gay, J.A. Boling, and R.B. Muntifering. 1987. Growth and metabolism of growing beef calves fed tall fescue haylage supplemented with protein and (or) energy. *J. Anim. Sci.* 65(4):1094-1100.

Spencer, L.A., and P.F. Weller. 2010. Eosinophils and Th_2 immunity: contemporary insights. *Immunol. Cell. Biol.* 88(3):250-256. doi: 10.1038/icb.2009.115

Stamm, M.M., T. Delcurto, M.R. Horney, S.D. Brandyberry, and R.K. Barton. 1994. Influence of alkaloid concentration of tall fescue straw on the nutrition, physiology, and subsequent performance of beef steers. *J. Anim. Sci.* 72:1068–1075.

Sterzl, J., Z. Rebacek, and J. Cudlin. 1987. Regulation of the immune response by ergot alkaloids. *Czech. Med.* 10:90-98.

Stocco, C., C. Telleria, and G. Gibori. 2007. The Molecular Control of Corpus Luteum Formation, Function, and Regression. *Endocrine Reviews* 28(1): 117-149. doi:10.1210/er.2006-0022

Stow, J.L., and R.Z. Murray. 2013. Intracellular trafficking and secretion of inflammatory cytokines. *Cytokine Growth Factor Rev.* 24(3):227-239. doi: 10.1016/j.cytogfr.2013.04.001

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.* 70:1228.

Strickland, J.R., J.W. Oliver, and D.L. Cross. 1993. Fescue toxicosis and its impact on animal agriculture. *Vet. Hum. Toxicol.* 35, 454–464.

Strickland, J.R., G.E. Aiken, D.E. Spiers, L.R. Fletcher, and J.W. Oliver. 2009. Physiological Basis of Fescue Toxicosis. Chapter 12; pp. 203-227. In: H.A. Fribourg, D.B. Hannaway, and C.P. West (ed.) Tall Fescue for the Twenty-first Century. Agronomy Monographs 53. ASA, CSSA, SSSA, Madison, WI.

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

Strosberg, A.D. 1993. Structure, function, and regulation of adrenergic receptors. *Protein Science* 2(8):1198-1209. doi: 10.1002/pro.5560020802

Sula, K., A. Fiserova, and M. Flieger. 1991. The preliminary results of ergot alkaloid role on the antibody production, delayed type of hypersensitivity, and oxidative burst of macrophages. *Proceedings 1st Symposium on Consuprene,* 50-54.

Takahashi, H., M. Takahashi, H. Nagaya, M. Hirako, K. Sawai, A. Minamihashi, S. Inumaru, Y. Yokomizo, M. Geshi, A. Okano, and K. Okuda. 2005. Establishment of a specific radioimmunoassay for bovine interferon tau. *Theriogenology* 63(4): 1050-1060.

Telford, N.A., A.J. Watson, and G.A. Schultz. 1990. Transition from maternal to embryonic control in early mammalian development: a comparison of several species. *Mol. Reprod. Dev.* 26:90-100.

Te Paske, M., and R. Powell. 1993. Analyses of selected endophyte-infected grasses for the presence of loline-type and ergot-type alkaloids. *J. Agric. Food Chem.* 41: 2299-2303.

Thatcher, W.W., P.J. Hansen, T.S. Gross, S.D. Helmer, C. Plante, and F.W. Bazer. 1989. Antiluteolytic effects of bovine trophoblast protein-1. *J. Reprod. Fertil. Suppl.* 37:91-99.

Trinchieri, G. 1989. Biology of natural killer cells. *Adv. Immunol.* 47:187-376.

Thompson, F.N., and J.A. Stuedemann. 1993. Pathophysiology of fescue toxicosis. Pp. 263-281. In: R. Joost and S. Quisenberry (ed.) *Acremonium* /Glass Interactions. Elsevier, Amsterdam, The Netherlands.

Turner, H.G., and A.V. Schleger. 1960. The significance of coat type in cattle. *Aust. J. Agric. Res.* 11(4):645-663.

Turner, H.G. 1962. Effect of clipping the coat on performance of calves in the field. *Aust. J. Agric. Res.* 13:180-192.

Turner, J. W. 1980. Genetic and biological aspects of Zebu adaptability. *J. Anim. Sci.* 50:1201-1205.

Turner, M.D., B. Nedjai, T. Hurst, and D.J. Pennington. 2014. Cytokines and chemokines: At the crossroads of cell signaling and inflammatory disease. *Biochim. Biophys. Acta.* 1843(11): 2563-2582. doi: 10.1016/j.bbamcr.2014.05.014

Turvey, S.E., and D.H. Broide. 2010. Innate immunity. *J. Allergy Clin. Immunol.* 125(2 Suppl. 2):24-32. doi: 10.1016/j.jaci.2009.07.016

Turzillo, A.M. and J.E. Fortune. 1990. Suppression of the secondary FSH surge with bovine follicular fluid is associated with delayed ovarian follicular development in heifers. *J. Reprod. Fertil.* 89:643-653.

USDA NASS, 2012 Census of Agriculture, Ag Census Web Maps.

Vasconcelos, J. L. M., R. Sartori, H. N. Oliveira, J. G. Guenther, and M. C. Wiltbank. 2001. Reduction in size of the ovulatory follicle reduces subsequent luteal size and pregnancy rate. *Theriogenology* 56(2):307-314. doi: 10.1016/S0093-691X(01)00565-9

Van Acker, H.H., A. Capsomidis, E.L. Smits, and V.F. Van Tendeloo. 2017. CD56 in the Immune System: More Than a Marker for Cytotoxicity? *Front. Immunol.* 8: 892. doi: 10.3389/fimmu.2017.00892

Visek, W.J. 1984. Ammonia: its effects on biological systems, metabolic hormones, and reproduction. *J. Dairy Sci.* 67(3): 481-498. doi: 10.3168/jds.S0022-0302(84)81331-4

Waldmann, T.A., S. Dubois, and Y. Tagaya. 2001. Contrasting roles of IL-2 and IL-15 in the life and death of lymphocytes: implications for immunotherapy. *Immunity* 14: 105-110.

Wallis, M. 1974. The primary structure of bovine prolactin. *FEBS Lett* 44:205-208.

Walls, J.R., and D.R. Jacobson. 1970. Skin temperature and blood flow in the tail of dairy heifers administered extracts of toxic tall fescue. *J. Anim. Sci.* 30:420-423.

Walner, B.M., N.H. Booth, J.D. Robbins, C.W. Bacon, J.K. Porter, T.E. Kiser, R. Wilson, and B. Johnson. 1983. Effect of an endophytic fungus isolated from toxic pasture grass on serum prolactin concentrations in the lactating cow. *Am. J. Vet. Res.* 44:1317.

Warrington, R., W. Watson, H.L. Kim, and F.R. Antonetti. 2011. An introduction to immunology and immunopathology. *Allergy, Asthma Clin. Immunol.* 7(Suppl 1):1. doi: 10.1186/1710-1492-7-S1-S1

Wassarman, P.M. 1992. Mouse gamete adhesion molecules. *Biol. Reprod.* 46(2):186-191.

Wassarman, P.M., D.F. Albertini. 1994. The mammalian ovum. In: The Physiology of Reproduction. pp. 79-122. Knobil, E., J.D. Neill (eds). New York: Raven Press.

Wathes, D.C., and G.E. Lamming. 1995. The oxytocin receptor, luteolysis and the maintenance of pregnancy. *J. Reprod. Fertil. Suppl.* 49:53-67.

Weaver, D.M., J.W. Tyler, D.C. VanMetre, D.E. Hostetier, and G.M. Barrington. 2000. *J. Vet Intern Med.* 14(6):569-577.

Wettemann, R.P., H.D. Hafs, L.A. Edgerton, and L.V. Swanson. 1972. Estradiol and progesterone in blood serum during the bovine estrous cycle. *J. Anim. Sci.* 34(6):1020-1024.

Wettemann, R.P. and H.A. Tucker. 1974. Relationship of ambient temperature to serum prolactin in heifers. *Proc. Soc. Exp. Biol. Med.* 146:908.

Whitman, R. W. 1975. Weight change, body condition and beef-cow reproduction. Ph.D. Diss. Colorado State Univ., Fort Collins.

Wilkinson, H.H., L.P. Bush, H.R. Burton, M.R. Siegel, and C. Schardl. 1997. Lolines are fungal metabolites. Poster Abstract 27. In: Poster Abstracts of the Int. *Neotyphodium*/Grass Interactions Symp. Athens, GA. 8-31 May 1997.

Williams, D.J., P.A. Blackman, M.A. Crawford, S.P. Schmidt, and C.C. King, Jr. 1984. Chemical control of the tall fescue endophyte and its relationship to cattle performance. *NZ J. Exp. Agric.* 12:165.

Williams, M.L., S.R. Shaffer, G.B. Garner, S.G. Yates, H.L. Tookey, L.D. Kintner, 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(9):1353-1357.

Wilson, T.B., M.R. Milnamow, M.A. West, D.B. Faulkner, F.A. Ireland, and D.W. Shike. 2014. Grazing novel endophyte-infected fescue following grazing endophyte-infected fescue to alleviate fescue toxicosis in beef calves. *J. Anim. Sci.* 92(E Suppl. 2).

Wiltbank, J.N. and L.E. Casida. 1956. Alteration of ovarian activity by hysterectomy. *J. Anim. Sci.* 15(1):134-140.

Wolfenson, D., B. J. Lew, W. W. Thatcher, Y. Graber, and R. Meidan. 1997. Seasonal and acute heat stress effects on steroid production by dominant follicles in cows. *Anim. Reprod. Sci.* 47:9-19.

Yates, S.G. 1962. Toxicity of Tall Fescue Forage: A Review. *Economic Botany* 16(4):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. *J. Agric. Food Chem.* 33:719-722.

Young, C.A., C.L. Schardl, D.G. Panaccione, S. Florea, J.E. Takach, N.D. Charlton, N. Moore, J.S. Webb, and J. Jaromczyk. 2015. Genetics, genomics and evolution of ergot alkaloid diversity. *Toxins* 7(4): 1273-1302. doi: 10.3390/toxins7041273

Zhang, J., and J. An. 2007. Cytokines, Inflammation and Pain. *Int. Anesthesiol. Clin.* 45(2): 27-37. doi:10.1097/AIA.0b013e318034194e

Zhu, D., T.L. Ott, and F.W. Bazer. 1996. Enzyme-linked immunosorbent assay for ovine interferon-tau. *J. Interferon Cytokine Res.* 16(2): 147-150.

Zhuang, J., M.A. Marchant, C.L. Schardl, and C.M. Butler. 2005. Economic Analysis of Replacing Endophyte-Infected with Endophyte-Free Tall Fescue Pastures. *Agron. J.* 97:711-716. doi:10.2134/agronj2004.0108

Appendices

Appendix A: MyBioSource Bovine Prolactin (PRL) Assay Validation

Table (Suppl. A1): A spike-and-recovery experiment was conducted in order to validate the Bovine Prolactin ELISA (MyBioSource, San Diego, CA). To certify the assay, the spike-and-recovery range should be 92-101% per manufacturer instructions. Samples were assayed by adding 150 µl of sample (25.53 ng/ml) and 150 µl of spike solution (standard diluent). Recovery for this assay was 96.5%.

Spike Level	Standard Diluent	Expected	Observed	Recovery
10 ng/ml	12.58 ng/ml	19.06 ng/ml	18.94 ng/ml	99.4%
12.5 ng/ml	16.17 ng/ml	20.85 ng/ml	21.41 ng/ml	102.7%
50 ng/ml	53.25 ng/ml	45.00 ng/ml	39.39 ng/ml	87.5%
				96.5%

Table (Suppl. A2): A linearity-of-dilution experiment was conducted to further validate the Bovine Prolactin ELISA (MyBioSource, San Diego, CA). To certify the assay, the linearity-of-dilution range should be 94-102% (1:2 dilution) and 92-104% (1:4 dilution) per manufacturer instructions. Manual suggests that samples should diluted either 1:2 or 1:4 and to avoid diluting samples more than 1:10. Multiple samples were serially diluted to measure the accuracy of this ELISA. Recovery for 1:2 dilution was 95.2% and for 1:4 dilution was 96.6%.

Recovery calculation of Linearity-of-dilution:

% change in concentration from previous dilution (neat) = $\frac{\boldsymbol{concentration\ (1{:}2\ dilution)}}{\boldsymbol{concentration\ (neat)}}\ \boldsymbol{x\ 100}\%$

% change in concentration from previous dilution (1:2) = $\frac{\boldsymbol{concentration\ (1{:}4\ dilution)}}{\boldsymbol{concentration\ (1{:}2\ dilution)}}\ \boldsymbol{x\ 100}\%$

Sample	Reading	Concentration	% Change
A (neat)	52.66 ng/ml	-	-
A (1:2)	25.77 ng/ml	51.53 ng/ml	97.9%
A (1:4)	11.35 ng/ml	45.40 ng/ml	86.2%
B (neat)	81.05 ng/ml	-	-
B (1:2)	42.06 ng/ml	84.12 ng/ml	103.8%
B (1:4)	21.38 ng/ml	85.52 ng/ml	105.5%
C (neat)	32.53 ng/ml	-	-
C (1:2)	13.23 ng/ml	26.45 ng/ml	81.3%
C (1:4)	8.65 ng/ml	34.61 ng/ml	106.4%
D (neat)	66.32 ng/ml	-	-
D (1:2)	32.36 ng/ml	64.72 ng/ml	97.6%
D (1:4)	14.64 ng/ml	58.56 ng/ml	88.3%

Appendix B: Embryo area and crown-rump length.

Table (Suppl. B1): Embryo area and crown-rump length for heifers beef heifers consuming either novel endophyte (EN) or endophyte-infected (EI) fescue with slick hair mutation (S) or wildtype (W) from late-May to late-August 2015.

	Treatment[1,2,3]				
Item[4]	**EN-S**	**EN-W**	**EI-S**	**SEM**	***P-value***
Embryo area, cm^2	3.99	3.83	4.17	0.74	0.944
Crown-rump length, cm	4.62	4.75	4.89	0.73	0.965

[1]Values are reported as least square means for the experiment
[2]EN-S: novel endophyte fescue with slick hair coat; EN-W: novel endophyte fescue with wildtype hair coat; EI-S: endophyte-infected fescue and slick hair coat
[3]No detection of pregnancy in endophyte-infected fescue with wildtype hair coat (EI-W) heifers.
[4]Values are averaged throughout the 58 to 88 d of the trial.

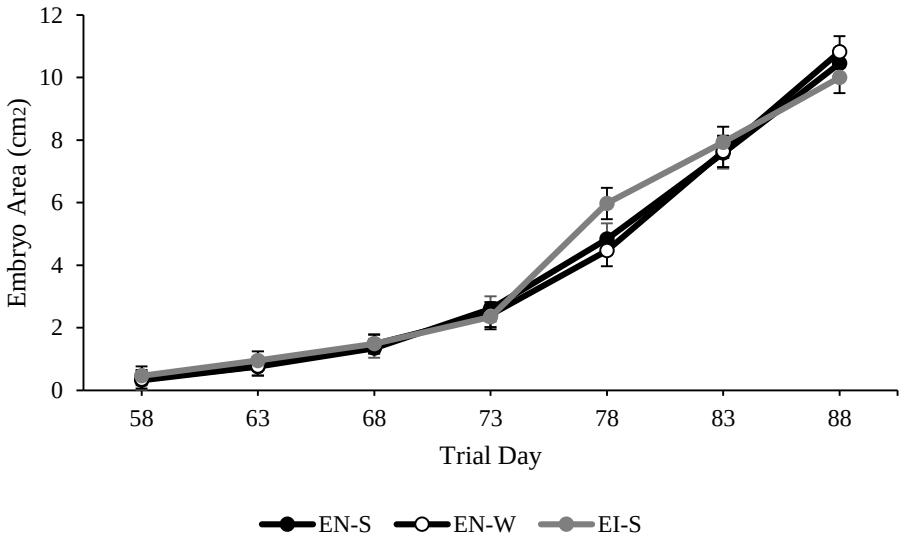

Figure (Suppl. B1): Embryo area over time for heifers beef heifers consuming either novel endophyte (EN) or endophyte-infected (EI) fescue with slick hair mutation (S) or wildtype (W) from late-May to late-August 2015. No detection of pregnancy in endophyte-infected fescue with wildtype hair coat (EI-W) heifers. For reference, d 58 is equivalent for d 30 of gestation. *P*-values: Time $P<0.0001$, Trt*Time $P=0.639$

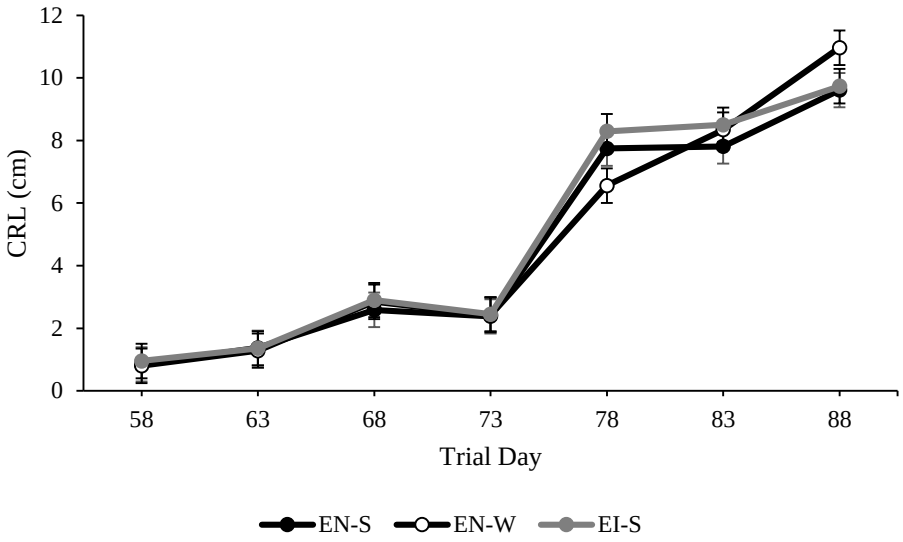

Figure (Suppl. B2): Crown-rump length (CRL) over time for heifers beef heifers consuming either novel endophyte (EN) or endophyte-infected (EI) fescue with slick hair mutation (S) or wildtype (W) from late-May to late-August 2015. No detection of pregnancy in endophyte-infected fescue with wildtype hair coat (EI-W) heifers. For reference, d 58 is equivalent for d 30 of gestation. *P*-values: Time $P<0.0001$, Trt*Time $P=0.328$